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PROCEEDINGS
OF THE
TWENTIETH MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

25 OCTOBER 1954

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Initial Distribution

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Twentieth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in the Council Chamber, National Research Building

9.30 a.m., October 25, 1954.

1. Attendance

Dr. J. B. Macdonald, Chairman

Dr. J. Aldous

Dr. J. M. R. Beveridge

Dr. A. C. Burton

Dr. D. H. Copp

Dr. A. W. Ham

Dr. C. D. Husband

Dr. L. A. Kilburn

Dr. J.-P. Lussier

Dr. E. Pagé

Brig. E. M. Wansbrough

Dr. J. B. Marshall, Secretary

Miss D. J. Wright

By invitation

Dr. E. E. Johns

Dr. R. L. deC. H. Saunders

Apologies for their absence were received from:

Dr. J. S. Bagnall

Dr. H. K. Brown

Dr. R. H. McDougall

Dr. A. G. Racey

Dr. C. H. M. Williams

2. Chairman's Remarks

THE CHAIRMAN welcomed to the Committee Dr. J.-P. Lussier, a new member representing the dental profession, and Dr. J. B. Marshall, the new Secretary; he mentioned that regrets for their inability to attend had been received from Dr. Bagnall, Dr. Williams, Dr. Brown, Dr. McDougall and Dr. Racey.

He also welcomed Dr. E. E. Johns of Kingston and Dr. R.L. deC. H. Saunders of Dalhousie University, two grantees who had been invited to be present and to report on their projects, and expressed the hope that they would feel free to take part in the discussions of the Committee.

He then read the following brief review of the Committee's operations during the nine years since its inception.

"This is the first meeting of the Associate Committee on Dental Research at which the total membership is composed of persons elected to membership subsequent to the formation of the Committee. It therefore seems worthwhile to review the record of the Committee over the past nine years.

"At the outset it should be stated that the Committee under the very able Chairmanship of Dr. R. G. Ellis has made remarkable progress in a few short years. Reference has been made by former President Mackenzie and President Steacie to the work of the Committee as a model example for other Committees. All who are interested in the promotion of dental research are indebted to Dean Ellis and the members of this Committee who have preceded us.

"The Committee was formed on March 16, 1945, and the first meeting was held on June 1st of the same year. At that time three main items were discussed:

"1. It was pointed out that personnel trained for or interested in dental research were almost non-existent. Practically no dentists with basic science training were resident in Canada and almost no biologic scientists were interested in dental research. It was considered of primary importance that this situation be remedied. In order to attract suitable dentists to research it was proposed that fellowships to the value of four to five thousand dollars be made available. At that time this amount was entirely out of line in relation to other fellowships offered by the National Research Council. The general conclusion was that efforts should be made to obtain suitable personnel but at that time it did not seem at all clear how this was to be accomplished.

"2. Considerable discussion evolved around the nature of the investigations which should be promoted. A list of some twenty proposed projects had been submitted to a conference on dental research late in 1944. The projects were mostly of a practical nature aimed at improving dental treatment services and relatively little attention was given to problems of prevention. The objective seemed to be one of deciding which problems were most important and then attempting to interest appropriate persons in carrying out the investigation.

"3. It was suggested that as a means of orienting the Committee and potential research workers, critical reviews should be prepared evaluating the state of knowledge and progress in the various fields of dental research. This point received great emphasis and was generally agreed upon.

"It is instructive to examine the progress which has been made in the light of these three points.

Personnel

"In the nine year interval the number of dentists trained for basic research has grown to approximately 12. In addition approximately 6 dentists are at present engaged in comprehensive training programmes. About 12 individuals with training in various basic sciences who do not hold dental degrees are conducting dental research projects. In a country of fourteen million this seems like a small number but compared to the situation existing nine years ago it represents a great advance.

"The slowest phase of growth is naturally associated with the training of qualified personnel. One might predict that growth should be geometric rather than arithmetic and that the next nine year period should see a great increase over the past nine years. N.R.C. fellowships have been increased in value to a maximum of \$5,000 which should be sufficient to attract more candidates than in the past. The greatest need at the present time is to develop research programmes in all the dental schools. Research at the University of Toronto and the University of Montreal appears to be making rapid progress but at the remaining three dental faculties the same difficulties exist that existed in 1945. The Canadian Dental Association is at the present time conducting a survey of the status of dental research in Canada and its report should be of considerable interest to this Committee in suggesting ways in which the difficulties of initiating adequate programmes can be overcome.

Problems

"In retrospect the Committee's attitude towards the selection of appropriate problems for investigation appears to have been unrealistic. A list of proposed projects was carried by the Committee for a number of years. No one used the list and indeed it would seem unlikely that any qualified individual would resort to such a list to decide what he wanted to do. Choice of problems in reality evolves around the choice of personnel. The kind and quality of training of individuals engaged in dental research plus their personal interests will determine the problem on which they choose to work. The Committee's interest in the problems being conducted by grantees might well be in terms of the following statement quoted from Dr. W. W. Woodbury at the first meeting of the Committee: "In the main, three problems face Dentistry, viz. caries, periodontal disease and malocclusion. We know very little about their etiology: hence preventive measures to date are most inadequate(these problems) will demand the combined wisdom of all the sciences represented by the members of the Committee." There is no doubt that the above philosophy has over the years become the policy of the Committee and it seems appropriate that it should continue to be so.

Reviews

"As the initial step in the preparation of critical reviews as proposed by Dr. D. Mainland at the first meeting, Dr. Bagnall agreed to prepare a critical survey of the literature on dental caries. Because of the nature of the subject Dr. Bagnall's work was much more than a review and in fact constitutes a monumental reference volume abstracting all the work on dental caries. However, no other reviews have been undertaken under the sponsorship of this Committee. It is my personal opinion that competently prepared critical reviews are of great value and that the Committee should consider further efforts at promoting their preparation. To be 'critical reviews' in the accepted sense of the term they must be limited in their scope. A subject such as dental caries does not lend itself to such treatment. Many subjects within the field of dental caries are eminently suitable (e.g. dental caries in experimental animals, bacteriology of dental caries, etc.) and many subjects could be chosen outside the caries field.

"I would like to suggest that the Committee consider the advisability of inviting qualified persons to prepare reviews under its auspices. If agreed upon in principle the reviewer might be invited to present his review in person at a Committee meeting in addition to publishing in a journal.

Summary

"It would appear that good progress has been made with respect to solving personnel problems for dental research but that efforts in this direction need to be continued and if possible expanded.

"The Committee's policy in respect to choice of projects for support has moved steadily in the direction of the support of basic science projects of direct or indirect significance to the three dental public health problems -- caries, periodontal disease and malocclusion. This should not limit the scope of investigations in basic science fields. The Committee should be prepared to support well-conceived basic studies whether or not they appear to have a relationship to public health problems.

"The Committee's one effort in the direction of preparing reviews has been well received and has been very useful for research workers throughout the world. Further efforts in this field are suggested."

DR. HAM congratulated the Chairman on the excellence of his review; he also questioned the fact that so few applications for grants in aid of research were received by the Committee.

DR. BEVERIDGE suggested that the reason lay in the scarcity of researchers trained in dentistry, and that the problem might be alleviated by encouraging graduate students in the basic sciences to undertake projects related either directly or indirectly to dentistry.

DR. BURTON pointed out that many researchers who might be working on problems of interest to the Committee were as yet unaware of the existence of the Committee and felt that wider publicity was needed.

DR. HAM suggested that the fact that funds are made available by the Committee for research be brought to the attention of the departments of basic science in the various universities, regardless of whether the university had a dental school.

DR. BURTON agreed with this suggestion and expressed the general opinion of the Committee when he added that if a greater number of applications were received the Committee could be more discriminatory and projects of definite value in the field of dentistry would gain the support they merit.

DR. HAM pointed out that it would be necessary in any publicity program to emphasize the high qualifications demanded by the Committee and the fact that applicants be able to show that they understand the application of their proposed projects to the field of dentistry.

The Committee felt that copies of the Chairman's Remarks to the Committee should also be sent to the basic science departments of the universities.

It was MOVED by Dr. Ham and SECONDED by Dr. Beveridge THAT the Associate Committee on Dental Research make known its existence and grant program to the basic science departments of all Canadian universities who might be interested in problems of significance to dentistry and who teach dental students, and that the Chairman's opening statement to the Committee be distributed with the publicity notice.

CARRIED.

THE CHAIRMAN agreed that he and the Secretary would prepare a statement which would be circulated to the members of the Committee for their approval before being distributed.

3. Minutes of the Nineteenth Meeting

It was MOVED by Dr. Pagé and SECONDED by Brig. Wansbrough THAT the Minutes of the nineteenth meeting be taken as read and approved.

CARRIED.

4. Business Arising out of the Minutes

THE CHAIRMAN noted that the only item of business arising from the minutes was the report of the action taken in connection with a patent application made by Dr. Nikiforuk as a result of his work on project DR-48 (Min. 16, 19th Mtg.).

THE SECRETARY reported that an application for a patent on a silicone preparation for the protection of teeth against acid erosion had been filed in the U.S. Patent Office on August 30, 1954, by the National Research Council's Patent Office. No further patents were being applied for at the present time. It is expected that the U.S. Patent Office will advise the Council by April or May, 1955, as to whether a patent will be granted. In any case, the Council's Patent Committee will consider next May the advisability of filing patent applications in countries other than the United States.

It was AGREED that no further action need be taken by the Committee at this time.

At the request of several members of the Committee, the SECRETARY outlined the work of the crown company, Canadian Patents and Development Limited, and the disposition of royalties obtained from patents. It was pointed out that one of the

reasons for obtaining patents on developments made by Canadian scientists was to make possible continued work on the projects concerned, unhampered by possible subsequent patent action taken by other workers in the same field.

5. Consideration of Reports from Grantees

At the meeting, the reports from grantees presenting their reports in person were considered first, and other reports were presented by members of the Committee as requested by the Chairman. For convenience of reference in the Proceedings, however, the reports are arranged in numerical order by project number. During presentation of the reports on his own two projects, DR-35 and DR-50, the Chairman asked Brig. Wansbrough to take the chair.

- i) DR-1: Dr. J. J. Rae
Reported by Dr. J. Aldous
"Chemical Mechanisms in Dental Caries".
A summary appears in APPENDIX H

Dr. Aldous admitted that he was somewhat confused as to the work being done by Dr. Rae since he understood that the work on ammonia production in saliva had been completed with the March report; it appears, however, to have been continued. He drew the attention of the Committee to apparent inconsistencies in Dr. Rae's reports; in March he stated that ammonia production appeared to be inhibited by glucose, whereas the October report indicates that ammonia production is stimulated by glucose. He noted too that in paragraph 1 of the October report, Dr. Rae states that "Wide variations in the ratios of urea and glucose had little effect on ammonia production", while in paragraph 2 he states that "A direct relationship was observed between urea content and ammonia production and between urea production and pH".

DR. BURTON questioned the value of this part of Dr. Rae's investigations since the present general opinion is that ammonia production is not a factor in eliminating dental caries.

DR. BEVERIDGE expressed the opinion that this type of work is essential, and pointed out that the problem is a difficult one because the reaction is not due to a property of pure saliva but of salivas from non-sterile oral cavities, which vary considerably.

After further discussion, the CHAIRMAN asked Dr. Aldous to draft a letter to be sent to Dr. Rae by the Secretary, requesting clarification of the apparent inconsistencies in his report. Dr. Rae's reply could then

be circulated to the members of the Committee with the report he submits for consideration at the next meeting.

It was AGREED that Dr. Rae should be asked to report on his project in person at the next meeting.

ii) DR-13: Dr. M. Doreen Smith

Reported by Dr. E. Pagé

"Fluoride content of dentin, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford".

A summary and report appear in APPENDIX J

Dr. PAGE, after presenting the report, noted the importance of producing the type of data provided by Dr. Smith and recommended that a statistical analysis of these data be made in order to make them a more useful basis for any action which might be taken in connection with the fluoridation of communal water supplies. This view was supported by DR. BURTON, who also pointed out the advisability of having a statistician who is familiar with the dental problems involved. The CHAIRMAN mentioned that Dr. Grainger, who has already co-operated with Dr. Smith in this project, would be well qualified to carry out such an analysis.

It was pointed out that a statistical analysis should be based on all data obtained to date, but the Committee realized that Dr. Smith's present grant was inadequate for this purpose; it was suggested that her next application include provision for a reasonable amount for such an analysis. It was AGREED that an estimate of the probable cost of statistical work of this kind should be obtained by the Chairman before the next meeting.

DR. PAGE questioned the meaning of the headings "Age" and "Length of Exposure" used in Dr. Smith's Tables. It was not clear whether these terms referred to the children involved or to the actual teeth studied.

THE CHAIRMAN asked Dr. Pagé to draft a letter to be sent to Dr. Smith by the Secretary, requesting clarification of the ambiguities and pointing out the desirability of statistical analysis of her data.

iii) DR-22: Dr. C. H. Best

Reported by Dr. A. W. Ham

"An Investigation of the Mechanism of Repair of the Supporting Structures of the Teeth".

A summary and report appear in APPENDIX G

DR. HAM read the summary submitted by Dr. Linghorne and elaborated on some of the experimental procedures for the benefit of the members of the Committee who were less familiar with the work being done under this grant.

DR. BURTON questioned the irregularity of this grant, which lies in the fact that the person paid under the grant, rather than the actual grantee, is responsible for the research. It was pointed out that this problem had been considered before (Min. 8, 18th Mtg.) and the CHAIRMAN read to the Committee the earlier comments, indicating that the present arrangement could only be regarded as temporary since it was contrary to the Council's policy concerning support of grantees. He pointed out that it would be to Dr. Linghorne's advantage, too, if the matter could be clarified and felt that every effort should be made to regularize the support given Dr. Linghorne under some aspect of the Council's award program.

DR. HUSBAND stated that Dr. Linghorne's work was of considerable importance to the profession and should be encouraged; he agreed with DR. HAM that the work would benefit from sympathetic control on the part of the Committee with a view to encouraging Dr. Linghorne to undertake and complete one project at a time.

DR. HAM suggested that the Division of Dental Research at Toronto apply for a consolidated grant under which Dr. Linghorne's salary could be paid. The CHAIRMAN, who is Director of that Division, agreed that this was a possibility to be considered but said that no application for a consolidated grant was being contemplated at the present time.

After further discussion, it was MOVED by Dr. Beveridge and SECONDED by Dr. Burton

THAT the Committee continue to receive annually applications from Dr. Best for grants in aid to support the work of Dr. Linghorne in the hope that a satisfactory and permanent solution to the problem of continuing financial support will be effected by appropriate action on the part of the Associate Committee on Dental Research in concert with other parties concerned. Until further notice, as an expression of confidence in the work of Dr. Linghorne, only annual reports on this investigation will be required.

CARRIED

- iv) DR-23: Dr. C. P. Leblond
Reported by Dr. A. W. Ham
"Entry of Phosphate, Carbonate and Calcium Ions into Teeth, as Shown with the help of Radioactive Elements".
The summary and report appear in APPENDIX L

After he had presented Dr. Leblond's report, DR. HAM stated that it was most impressive and, together with the work being done by Dr. Bélanger and Dr. Saunders, represented

work of great value to a fundamental understanding of the tooth.

- v) DR-28: Dr. O. J. Walker
Reported by Dr. C. D. Husband
"Investigation of Methods for Destroying the Organic Matter in Teeth, Bones, and other Materials Prior to Determination of Fluorine Content".

A summary of the work appears in APPENDIX I

DR. HUSBAND reviewed the earlier work under this project relating to a rapid and successful method of ashing dental material in preparation for fluorine determinations. The CHAIRMAN pointed out that no financial assistance was necessary since the colorimeter had been reconditioned. DR. HUSBAND indicated that Dr. Walker expected to present his final report on this project to the next meeting.

- vi) DR-35: Dr. J. B. Macdonald
Reported by himself
"Bacteriology of Periodontal Disease. I. Experimental Fusospirochetal Infection with Mixtures of Pure Culture".

A summary and report appear in APPENDIX D

After presenting his report, Dr. Macdonald mentioned that a paper on the work is expected to appear shortly in the Journal of Infectious Diseases. He also mentioned that the experiments are being duplicated in the laboratories of the St. Louis Jewish Hospital and he hopes that these further independent findings will support his own.

DR. BEVERIDGE commented on the complexity of the mechanism of infection where a group of organisms is involved and Dr. Macdonald agreed that an understanding of the mechanisms involved in this mixed infection would be a most valuable contribution and a highly interesting study.

DR. ALDOUS pointed out that Dr. Macdonald's results are of great interest from the point of view of chemotherapy.

- vii) DR-37: Dr. H. Jenkins
Reported by Dr. Macdonald
"Further studies in the Electromyography of the Head, Face and Neck".

A summary of the work appears in APPENDIX B

DR. MACDONALD read the summary of the work submitted by Dr. Jenkins. DR. JOHNS mentioned that he had seen Dr. Jenkins recently and that about half the samples (20) have now been collected. Dr. Scott of the Sick Children's Hospital is assisting with the collection of cephalometric data. The study is concerned with the relation of muscles to occlusions and facial development.

- viii) DR-43: Drs. S. G. Davis and H. R. MacLean
Reported by Dr. C. D. Husband
"Electropotentials of dental alloys".
A summary of this work appears in APPENDIX M

The work on this project is proceeding very slowly due to other demands on the time of Dr. Davis. As time permits, the grantee is measuring the potentials between various commercial dental alloys; these are in the order of 10 - 45 millivolts. In reply to the Chairman's question, DR. BURTON stated that such levels would be sufficient to affect tissue cells, etc. He also pointed out that he would like to see the investigation carried from a purely physicochemical study in vitro to an investigation of fillings in actual teeth.

DR. HUSBAND reported that Dr. Davis hopes to do some work on insulators of amalgams, working at various pHs and with various silver dental alloys.

- ix) DR-50: Dr. J. B. Macdonald
Reported by himself
"The Cultivation and Characteristics of Bacteroides nigrescens".
A summary and the report appear in APPENDIX E

DR. MACDONALD stated that while this project had originally been undertaken as a short-term project to fill a gap in the research program, it had developed into a problem requiring solution before the main project could be fully elucidated. He stated that the "growth factor" which had been found to be heat stable and apparently time stable for about two weeks, might be a polypeptide; he pointed out that polypeptides are used as such by some organisms.

- x) DR-51: Dr. R. L. deC. H. Saunders
Reported by himself
"Study of Human Dental Pulp Blood Vessels by X-ray Microarteriographic Methods".
A summary of this work appears in APPENDIX O

DR. SAUNDERS expressed his thanks to the members of the Committee for inviting him to be present; he also acknowledged the great measure of assistance he had received from Mr. A. E. Hoffman, a 4th year dental student, in the capacity of research assistant.

DR. SAUNDERS gave a most informative outline of his work in connection with the tracing of the vascular pattern in dental pulp, illustrated by a series of excellent slides of microarteriograms. He emphasized the difficulty of getting a complete picture of human pulp vessels and pointed out that the existing literature on the subject is chiefly

conjectural. Dr. Saunders has developed the use of radiopaque materials to clarify the subject and has achieved most enlightening results with this technique.

DR. HAM complimented him highly on the quality of his work and the excellence of his illustrative material.

DR. BURTON expressed the hope that his work is the forerunner of further physiological and anatomical studies of teeth and suggested that the investigations be extended to in vivo experiments.

DR. SAUNDERS stated that Mr. Hoffman, after graduation next spring, hopes to be able to continue working on the project.

THE CHAIRMAN asked if any attempt had been made to use chelating agents for decalcification; Dr. SAUNDERS said that no such attempt had been made, and the CHAIRMAN drew his attention to recent work by Nikiforuk and Sreedny (J. Dent. Res., 32:859, 1953).

In reply to DR. BEVERIDGE's question as to whether dilating agents were effective, DR. SAUNDERS said that it was too soon to say, and added that the problem of fluid expansion in enclosed space was a very acute one.

THE CHAIRMAN thanked Dr. Saunders for his admirable presentation of the work being carried on and said that remarkable progress was obviously being made in his laboratories.

- xi) DR-52: Dr. L. F. Bélanger
Reported by Dr. D. H. Copp
"Mechanism of Bone and Tooth Formation in the Light of Histochemistry and Autoradiography".
A summary of the work appears in APPENDIX C

DR. COPP stated that the work done since the last meeting has not been very extensive due to Dr. Bélanger's ill health and the change in location of his laboratory at Ottawa University. He stressed the high qualifications of Dr. Bélanger, in which he was enthusiastically supported by DR. BURTON, and mentioned that Dr. Bélanger would prefer to make a more complete report at the next meeting.

It was AGREED that Dr. Bélanger be invited to report in person on this project at the next meeting of the Committee.

- xii) DR-54: Dr. B. N. Kropp
Reported by Dr. A. C. Burton
"Development of a rapid method for grinding thin sections of plastic-embedded teeth".
A summary of the work appears in APPENDIX K

DR. BURTON questioned the purpose of Dr. Kropp's work in developing a method of cutting six sections of a tooth simultaneously but was assured by both DR. HAM and DR. SAUNDERS that the process would be most useful in the preparation of good teaching sections and should prove to be an important research tool.

DR. BURTON suggested that any further assistance given in connection with this project should be in relation to the application of the equipment developed thus far.

THE CHAIRMAN read a letter from Dr. Kropp indicating that he considered the device patentable. It was AGREED that when the final report is received by the Secretary it should be referred to the Council's Patent Office for investigation of possible patentability.

- xiii) DR-55: Dr. M. R. Culbert
Reported by Dr. J. B. Macdonald
"A Cephalometric Study of Maxillary Growth as
Related to Statural Growth".
A summary of the work appears in APPENDIX N

DR. MACDONALD felt that no comment on this work was necessary at this time and indicated that a final report would probably be forthcoming at the next meeting. He pointed out that more extensive knowledge of the times and rates of growth of the maxillae and long bones was of considerable importance from the standpoint of orthodontic treatment.

- xiv) DR-56: Dr. D. J. E. Mitchell
Reported by Dr. L. A. Kilburn
"A Comparative Study of Palatal Height, Width
and Length in Ancient and Modern Crania".
A summary of the work to date appears in APPENDIX A

After DR. KILBURN had read the report to the Committee, DR. JOHNS mentioned that he and Dr. Mitchell had visited New York together and worked in conjunction with each other to prevent waste of time in examining the large number of crania samples available. DR. JOHNS also said that he understood that in addition to measuring the length and width of the palate, as noted in the summary, Dr. Mitchell has measured the actual height of the palate in order to determine the type of malocclusion in the crania examined. It is hoped that these measurements can be further supplemented this fall by data available from Washington.

- xv) DR-57: Dr. G. Nikiforuk
Reported by Dr. J. M. R. Beveridge
"The Amino Acid Composition of Enamel Protein
Using Paper Chromatography".
A summary and report appear in APPENDIX F

DR. BEVERIDGE reported that the grantee has been fairly successful in the purification of enamel by first drying the teeth, then chipping off the enamel and separating the enamel and dentin by taking advantage of their difference in specific gravity. He questioned the meaning of the term "junction particles" and the CHAIRMAN explained that these were particles composed of both enamel (about 9/10) and dentin (about 1/10). DR. BEVERIDGE suggested that the drying procedure followed might be reviewed; he pointed out that the longer one subjects protein to heat the greater its degradation, and above 60°C a progressive degradation occurs. For this reason he suggested vacuum drying at lower temperatures.

To DR. PAGE's inquiry as to the accuracy of the separation, the CHAIRMAN replied that the best accuracy which has been obtained is .1 - .2% dentin, and added that corrections can be made on the basis of data which is available on the organic content of dentin.

THE CHAIRMAN stated that Dr. Nikiforuk intends to apply chelating agents to the decalcification. He said that the grantee was proceeding carefully with the application of paper chromatographic technique.

The members of the Committee felt that this problem was being attacked with industry and intelligence.

- xvi) Dr. Johns' travel grant
Reported by himself

A brief account of the presentation appears in APPENDIX P

Dr. JOHNS expressed his pleasure at being invited to report to the Committee on his work in connection with a study of the development of occlusions on primitive diets. This work was started three years ago by Dr. Moyers who had received a grant from the U.S. National Research Council to visit Greece in order to follow up the earlier studies made on his own initiative while he was in that country with the Army.

DR. JOHNS outlined the results of the survey made in Greece and showed the Committee a series of slides to illustrate his points. He described his subsequent work in this connection since his return to Canada, the control group

set up in Burlington and results obtained with experimental animals.

DR. BURTON asked if the Eskimos would be useful for study in this connection, and DR. JOHNS stated that the records and study models made of Eskimos by Dr. Williams in 1939 were available to him, and gave evidence of the same phenomenon as was apparent in the children studied in Greece.

DR. BEVERIDGE suggested that monkeys might be used experimentally; DR. JOHNS reported that Dr. Williams had used rats, since they were cheaper and gave more rapid results, and had found that vigorous chewing resulted in larger arches.

THE CHAIRMAN thanked Dr. Johns for his presentation of the report and expressed the interest of the Committee in his subsequent findings.

6. Finances

At the request of the Chairman, the SECRETARY presented the financial statement of the Committee as of 15 October 1954:

Allotment	40,700.00
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Expenditures

Awards - Grants	23,345.00
Fellowships	<u>6,000.00</u>
	29,345.00

Miscellaneous

Travel	242.70
Duplication	36.89
Reprints	<u>12.64</u>
	<u>292.13</u>
	29,637.13

Unliquidated Encumbrance	<u>11,062.87</u>	40,700.00
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It was pointed out that after deducting the costs of the 20th and 21st meetings of the Committee from the present balance of slightly over \$11,000, it appeared possible that an unexpended balance of about \$8,000 would be available.

At this point, DR. BURTON raised the question of whether the Committee should allocate \$4000 - \$5000 towards the cost of a symposium on fluoride in relation to dental caries, bringing workers from both Canada and other countries

together for a scientific consideration of the subject, the findings to be published following the conference.

THE CHAIRMAN stated that the Canadian Dental Association has reviewed the whole subject and published a review for public consumption.

DR. LUSSIER mentioned the symposium on the subject published by the American Association for the Advancement of Science in 1944.

THE CHAIRMAN suggested that the matter be deferred for consideration at its next meeting when Dr. Brown as an expert in the field would be present to express the views of the Department of National Health on the subject.

DR. PAGE mentioned that the dental school of one of the Canadian universities might sponsor such a symposium if it could obtain financial assistance from the Council.

DR. BEVERIDGE felt that in view of the general interest in the question at the present time, it was strange that no requests for information had been received by the Committee, but the CHAIRMAN suggested that this too was further evidence that the Committee had not made itself widely enough known.

DR. HAM suggested that such a conference might lead to a somewhat embarrassing press problem, since the Committee might conceivably be censured for appearing to neglect the question. He suggested that a survey be made to determine what research is now being carried out in Canada on the subject of fluorine in relation to dental caries, bone changes, incidence of periodontal disease, etc.

DR. BURTON felt that such areas of activity were the responsibility of the Committee and should not be avoided because of the possibility of some public censure.

DR. BEVERIDGE suggested that a critical review of the desirability of fluoridation of water - both human and animal studies - be made.

THE CHAIRMAN pointed out that several up-to-date reviews are already available and after some further discussion it was AGREED that the Secretary should distribute to the members of the Committee copies of three such reviews, as chosen by the Chairman, and that the subject of a possible symposium on the problem be placed on the agenda for the next meeting.

7. Authorization of Special Travel Grants

THE CHAIRMAN reminded the members of the Committee that last year the Executive had been authorized to make four travel grants to grantees working on projects sponsored by the Committee.

It was MOVED by Dr. Husband and SECONDED by Dr. Kilburn THAT the Executive be empowered to make up to four special travel grants this year to enable grantees to present papers at scientific meetings.

CARRIED

8. Requests for Supplementary Grant

THE CHAIRMAN read to the Committee a request from Dr. Nikiforuk for an additional grant of \$300 to be spent on supplies needed in connection with project DR-57.

It was MOVED by Dr. Beveridge and SECONDED by Dr. Aldous THAT the additional amount of \$300 requested by Dr. Nikiforuk be granted.

CARRIED

9. Fellowships

At the Chairman's request, the SECRETARY reported that a change had been made in connection with the fellowship granted to Dr. Reynolds. Dr. Reynolds is no longer working in the University of Toronto's Department of Pharmacology but in the Department of Biochemistry under Dr. B. Crocker; no other change in his program has taken place.

10. Membership

THE CHAIRMAN reported that Dr. W. S. Hartroft, who had been elected to membership on the Committee at the 19th Meeting, had left Canada to accept a position as head of the Department of Pathology of Washington University at St. Louis, and had therefore resigned from the Committee. He pointed out that it would be desirable to have a pathologist on the Committee, and possibly one from some Ontario university to maintain the geographic balance of the membership.

After some discussion, it was MOVED by Dr. Ham and SECONDED by Dr. Beveridge THAT Dr. H. J. Barrie of the Department of Pathology of the University of Toronto be nominated to membership on the Committee to fill out the unexpired portion of Dr. Hartroft's term.

No further nominations were received, so Dr. Barrie was elected to the Committee by acclamation, pending approval of Council.

11. Publications by Grantees

i) Reprints received since last meeting:

THE SECRETARY reported that reprints of seven papers published as a result of grants made by the Committee had been received and numbered in the List of Publications of the Associate Committee on Dental Research; the titles of the papers, which are numbers 28 to 34 in the list which appears in APPENDIX Q, were read to the Committee.

ii) Reprints to be held in stock by Secretary:

There was some discussion as to the number of reprints to be provided for the Secretary's stock and for distribution on request. At present, 25 reprints are purchased by the Committee and kept on file for the possible use of Committee members or others who may request them. Since very few requests for these papers are received by the Secretary, it appears to serve no useful purpose to have this stock lying dormant when the reprints might be put to better use by the authors.

It was AGREED that copies of reprints of those papers published since the last meeting be distributed to the members of the Committee, and new reprints be distributed as they are received.

DR. BEVERIDGE raised the question of why the Council did not pay, on behalf of the authors, the cost of reprints of papers published by researchers working on Council grants. He pointed out that the additional cost to the Council would be small in relation to the amount expended in support of the research yielding the published results, and would save the authors personal expense which in many cases they could ill afford.

After discussion, it was MOVED by Dr. Copp and
SECONDED by Dr. Beveridge

THAT the cost of up to 100 reprints of papers published in the scientific literature be considered a legitimate charge against a grant.

CARRIED

The Secretary was asked to bring this motion to the attention of Council for a decision as to the policy to be followed.

12. Report on Distribution of Dr. Bagnall's Bibliography on Caries Research

THE SECRETARY reported that 9 copies of the Bibliography had been sold since the last meeting, bringing the total distribution of the book to 270. 47 copies remain in stock.

THE CHAIRMAN noted that at the 19th Meeting (Min. 33) some consideration had been given to the possibility of preparing a supplement on relevant material published since the Bibliography was completed in 1950. It was AGREED that no further action be taken at this time, pending an expression of opinion from Dr. Bagnall as to his willingness to undertake the preparation of such a supplement.

13. New Business

1) Application for a grant from Dr. H. J. MacConnachie: THE CHAIRMAN read to the meeting an application from Dr. H. J. MacConnachie of the Faculty of Dentistry of Dalhousie University for a grant of \$5,503 to carry out a study to determine a method of utilizing the clinical material from the pre-natal clinic of the Dalhousie Public Health Centre for the purpose of evaluating the role of dental infection. The application was supported by Dr. McLean, Dean of Dentistry of Dalhousie, who felt that the institution of a treatment program would reveal a use for the vast amount of clinical material which had been obtained. The CHAIRMAN read to the Committee copies of correspondence which he had had with Dean McLean, in which it was suggested that for a pilot study a treatment program was not justified.

DR. ALDOUS, who was familiar with the intentions of the applicant, suggested that Dr. MacConnachie be given a fellowship to study the possibilities of the problem. The SECRETARY stated that it would be contrary to Council policy to grant a fellowship for such a program.

DR. SAUNDERS, when asked for his opinion, said that although he was not too familiar with the proposed project, he felt that it deserved the encouragement of the Committee, particularly if some more concrete objective could be decided upon.

DR. COPP suggested that the mothers' teeth should be followed up after the birth of the children, rather than limiting the study to the teeth of the children of women whose dental background was known.

Considerable discussion of the matter ensued, during which the Committee expressed itself as sympathetic to the application and the desire to use the material obtained at the Clinic.

It was MOVED by Dr. Kilburn and SECONDED by Brig. Wansbrough THAT the application be not granted at the present time, but that Dr. Saunders and Dr. Aldous discuss it further with Dean McLean and

Dr. MacConnachie in order to arrive at a more concrete proposal for making use of the clinical material.

CARRIED

In connection with the above application, it was the feeling of the Committee that the rate of pay of \$20 per half day proposed for Dr. Faulkner was considered to be high in comparison with rates paid to fellowship holders and others for the same type of work. It was suggested that this opinion be conveyed to Dean McLean by Dr. Saunders and Dr. Aldous in the course of their discussions.

14. Date of Next Meeting

THE CHAIRMAN suggested that no date be set at the present time, since it would be convenient to some of the members of the Committee to have the spring meeting immediately prior to or following the spring meeting of the Associate Committee on Medical Research, the date of which had not yet been determined. A suitable date will be set later by the Chairman and Secretary.

The meeting adjourned at 5:35 p.m.

APPENDIX A

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: A comparative study of palatal height, width and length in ancient and modern crania.

Grantee: David J.E. Mitchell,
University of Toronto.

Project No.: DR-56

Amount of Grant: \$400.00

SUMMARY OF WORK

During the month of April 1954, the collection of human crania at the New York Museum of Natural History was examined. The collection, which comprises some 12,000 specimens, was screened by the use of an accurate catalogue to determine which groups were composed of bone fragments and which contained complete crania. Of these 463 were examined and measured, thus permitting the sample to include the following racial groups:

Australian (Aboriginal), Chinese (Malay Pehinsula, Singapore), North and Central Europe (Hungarian, Austrian, Bulgarian, Danish), Negroes (South Pacific, Polynesian), (East African, Mombasa, Kipini).

The following measurements were taken (from Ales Hrdlicka - Practical Anthropometry)

1. Length of the palate - the distance between the following landmarks - anteriorly the median point of a line tangent to the posterior alveolar border of the central incisors; posteriorly the median point of a transverse line connecting the most anterior points of the notches in the posterior border of the palate.

2. Breadth of the palate - distance between the internal alveolar borders between the second molars.

From the accrued data the Palatal Index was computed and an attempt was made to classify the groups as:

Leptostaphyline
Mesostaphyline
Brachystaphyline

The measurements were treated to determine the mean and standard deviation for each group.

Unfortunately the sample sizes were not as great as was anticipated. This was largely due to the fact that the majority of the specimens were fragmented to varying extents. It is for this reason that the grantee has made arrangements to visit the collection of human crania in the Smithsonian Institution (Washington, D.C.) which is under the jurisdiction of Dr. J. Stewart.

Grantee: David J. E. Mitchell
University of Toronto

Project No.: BR-56
Amount of Grant: \$400.00

SUMMARY OF WORK

During the month of April 1954, the collection of human crania at the New York Museum of Natural History was examined. The collection, which comprises some 12,000 specimens, was screened by the use of an accurate catalogue to determine which groups were composed of bone fragments and which contained complete crania. Of these 463 were examined and measured, thus permitting the sample to include the following racial groups:

Australian (Aboriginal), Chinese (Mainland, Singapore), North and Central Europe (Hungarian, Austrian, Bulgarian, Danish), Negroes (South Pacific, Polynesian), East African, Mongolian, Kiplin).

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2. Breadth of the palate - distance between the internal alveolar borders between the second molars.

From the secured data the Palatal Index was computed and an attempt was made to classify the groups as:

Leptostaphyline
Mesostaphyline
Brachystaphyline

The measurements were treated to determine the mean and standard deviation for each group.

APPENDIX B

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Further studies in the electromyography of the head, face and neck.

Grantee: Dr. D. H. Jenkins,
University of Toronto.

Project No: DR-37 Amount of Grant: \$225.00

SUMMARY OF WORK

Re: Project of 1953-54

A precis of the work done by Doctors Street and Cook was published in the J.C.D.A. 20: 438-439, August 1954.

Re: Project of 1954-55

To date the sample of forty children has been assembled. Arrangements have been made at both the Hospital for Sick Children and the Western Hospital for assembly of models, cephalographic and electromyographic records.

We have been awaiting the arrival, from England, of an important photographic accessory to the electromyographic machine since June. It is expected that this will be installed and operating by October 1st.

Part 3

A considerable amount of material (bones and teeth) has been received from the Oak Ridge Fluoride project during the summer but could not as yet be processed and studied due to the conditions mentioned in part 2.

APPENDIX C

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Mechanism of bone and tooth formation in the light of autoradiography and histochemistry.

Grantee: Dr. L.-F. Bélanger,
University of Ottawa.

Project No.: DR-52

Amount of Grant: \$3,470.00

SUMMARY OF WORK

Part 1

Calcium Intake in Vitro: The cartilage has been extensively studied. The results submitted for publication indicate that the in vitro intake of Calcium is proportional to the amount of sulfated polysaccharide (chondroitin sulfate, mucoitin sulfate) in any one given tissue. This amount has been seen to decrease with age; it decreases previous to mineralization and it is also seen to decrease in the cartilage of humans suffering from rickets of variable duration.

Part 2

Two series of young rats have been injected with S³⁵ Cystine and S³⁵ Methionine respectively. The autoradiographic treatment was completed up to the phase of staining and mounting before the department was removed in June to its present quarters in the new medical building. There has been an unfortunate period of stagnation lasting from June until now due to the absence of organization of the new quarters and also to a surgical operation undergone by the Grantee. Conditions are coming progressively to normal now and it is hoped that this material will be studied within the next two months.

Part 3

A considerable amount of material (bones and teeth) has been received from the Oak Ridge Fluorine project during the summer but could not as yet be processed and studied due to the conditions mentioned in part 2.

APPENDIX D

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Bacteriology of periodontal disease.
I. Experimental fusospirochetal infections
with mixtures of pure cultures.

Grantee: Dr. J. B. Macdonald,
University of Toronto.

Project No.: DR-35 Amount of Grant: \$2,300.00

SUMMARY OF WORK

The pathogenicity of an experimental fusospirochetal exudate has been investigated in terms of the minimum combination of organisms capable of producing typical guinea pig infections. From a total of 17 organisms isolated in pure culture only 4 were required to produce typical lesions. The 4 essential organisms included 2 strains of Bacteroides (one of which was identified as Bacteroides nigrescens), a motile gram-negative anaerobic rod, and a diphtheroid. Spirochetes, fusiforms, vibrios and anaerobic streptococci were found to be non-contributory.

The accompanying report is the first draft of a paper dealing with this study.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Bacteriology of periodontal disease. I. Experimental fusospirochetal infections with mixtures of pure cultures.

Grantee: Dr. J. B. Macdonald,
University of Toronto.

Project No.: DR-35

Amount of Grant: \$2,300.00

PROGRESS REPORT

"The pathogenic components of an experimental fusospirochetal infection"

J.B. Macdonald, R.M. Sutton, M.L. Knoll and E.M. Madlener
Division of Dental Research, University of Toronto.

It was recently shown that infections having all the attributes of experimental fusospirochetal infections (Rosebury and Foley, 1939) could be produced regularly in guinea pigs with a combination of 17 different organisms -- all isolated in pure culture from a guinea pig mixed infection (Macdonald, Sutton and Knoll, 1954). Of the 17 organisms 15 were strict anaerobes and included streptococci, Bacteroides, spirochetes, vibrios and other motile rods, and non-motile gram-positive rods, presumably diphtheroids; two were facultative and included a strain of Streptococcus mitis and a diphtheroid.

A new technique of recombination, the "wheel-plate" technique was employed in the successful recombination. This technique consisted of inoculating a blood agar medium on Petri plates with each of the pure cultures as a single streak starting at the edge of the plate and finishing in the centre in such a way that on completion the streaks appeared as spokes of a wheel. After incubation each streak could be examined for evidence of pure growth of the inoculated strain and the hub of the wheel in successful plates contained mixed growth of all the organisms.

In the present study the mixed infection has been further analyzed by deleting organisms from the recombinations to determine the minimum combination capable of eliciting typical guinea pig responses.

Elimination of Non-Essential Organisms

Method:

Wheel-plates were prepared in which various organisms were omitted, and guinea pigs were inoculated with mixed growth obtained by subculture from the wheel-plates following the same procedure as in the recombination of 17 organisms previously described (Macdonald, Sutton and Knoll, 1954). Each recombination emulsion was inoculated into separate groups of four guinea pigs; 1 ml. per dose. In those instances in which infection resulted, exudate was aspirated (usually from two of the four animals) and passed to additional animals in a dosage of 0.1 ml. In this way the infection was carried through four or more passages wherever possible. The lesions in all guinea pigs were scored from 0 to ++++ according to the procedure recorded in Table 1.

In initial experiments recombinations were prepared in which all strains within a morphologic group were omitted.^{*} Subsequently individual strains within groups which appeared essential were omitted.

The results were evaluated by analyzing for two attributes -- infectivity and transmissibility. Infectivity was considered to depend on the ability of the mixed culture to produce infection in the first passage. The occurrence of infection in passages beyond the first was considered to be a measure of transmissibility. For each attribute the proportion of failures ($0, \pm$) to the total number of animals inoculated in the experimental group was compared to the corresponding proportion in a control group. The control for this purpose was a group of 60 animals inoculated with the total recombination mixture in three separate experiments (Macdonald, Sutton and Knoll, 1954). The method did not take into consideration the severity of infection as indicated by the scoring + to ++++, but analysis of the results of inoculating 134 guinea pigs with unpurified HG exudate indicated that there was no significant relationship between the occurrence of failures ($0, \pm$) and the severity of the infection from which the inoculum was derived in the range + to ++++. 38 guinea pigs failed to develop infection when inoculated with exudate from localized lesions as against 61 which developed typical infection. Using exudate from spreading lesions 8 guinea pigs failed to develop infection and 27 developed typical infection.

^{*} Strain K100, a streptococcus was omitted from all recombinations because earlier studies showed it to be unnecessary.

Results:

The results are tabulated by groups arranged alphabetically as shown in Table 1. The findings in groups A to C inclusive suggested that spirochetes, cocci, and fusiforms could be omitted without interfering with the infectivity or transmissibility of the infection as judged by the proportion of typical infections in each group. The findings for these three groups were confirmed in subsequent groups -- i.e. groups J, K, N, Q, and W. In only one group was there apparent conflict with this result -- i.e. group G in which there appeared to be a significant loss in transmissibility. In view of the repeated confirmations this discrepancy was regarded as non-significant and possibly related to fluctuation of the numbers of organisms in the constant volume inocula.

The results in groups D to F suggested a need for representatives of the gram-positive rods, the motile rods, and the Bacteroides. In groups H to J the three strains of Bacteroides were omitted singly along with spirochetes and cocci. Losses in infectivity and transmissibility occurred in the absence of either K110 (Bacteroides nigrescens) or K92, but not in the absence of JR6. The dispensability of the latter was confirmed in groups K, N, Q and W.

Among the gram-positive rods, strain JB3B appeared to be dispensable (group N). Omission of JB3A (group M) seemed to result in a loss of transmissibility but this finding was not confirmed in groups Q and W. Omission of JR3 (the facultative diphtheroid) affected transmissibility (group L). When JR3 was omitted from wheel-plates with smaller combinations than in group L, strain K110 (Bacteroides nigrescens) consistently failed to grow. By cross-streaking K110 with JR3 it was found that K110 grew as a satellite of JR3. The need for an unidentified 'growth factor' for strain K110 was reported previously (Macdonald, Sutton and Knoll, 1954). Cross-streaking strain K110 against all of the strains disclosed that in the presence of strains JR3, JR4, JR5 or JS9, strain K110 grew well. It was found to grow poorly in the presence of strain JB3B and not at all with any of the other strains. It therefore appears that one function of strain JR3 in the mixed infection was to support growth of strain K110.

* Analyzed by determination of normal deviate for difference in experimental proportion from expected. $Z = \frac{p_1 - p_2}{\sqrt{P(1-P)(1/n_1 + 1/n_2)}}$

where p_1 equals the experimental proportion, p_2 the expected and P is the weighted average. The probability value for a normal deviate of the size calculated was derived from a table of areas under the normal curve.

Omission of the two strains of motile rods singly in groups O and P suggested that both were needed for typical infections. However in subsequent combinations of the remaining five strains, four at a time, it was shown that one of them, JV5, could be omitted without evidence of change in infectivity or transmissibility.

The results of these elimination studies suggested that a combination of not less than four organisms was essential to the production in the guinea pig of mixed infection comparable to the 'fusospirochetal' infection produced with 17 organisms. The four strains apparently essential included two strains of *Bacteroides* (K110 and K92), one motile gram-negative rod (K80) and an aerobic diphtheroid (JR3).

Comparative Pathogenicity of Three Recombination Mixtures

On the basis of the above findings it was postulated that a combination of the four apparently essential organisms should produce infection in guinea pigs indistinguishable from that produced by the total group of 16^{*} strains; and that the total group without the 4 essential organisms should be non-pathogenic. This hypothesis was tested. For convenience the above 3 recombinations are referred to as "Four" (the essential 4, see Table 1), "Sixteen" (for total) and "Twelve" (total less the essential 4 strains).

Method: Wheel-plates were prepared for the "Four" group and the "Twelve" group. The wheel-plate growth was emulsified in glass tissue grinders and subcultured as unstreaked drops to fresh blood agar in each instance. The growth from 12 drops of each was emulsified in 6 ml. of broth. Three ml. of the resultant emulsion of "Four" were mixed with 3 ml. of the emulsion of "Twelve". This procedure mechanically reconstituted the total group of 16 organisms and was labelled "Sixteen". Three ml. of the remaining "Four" emulsion were emulsified with 3 ml. of an emulsion of sterile blood agar and broth prepared in the same way as the "Four" and "Twelve" emulsions. A similar mixture was prepared from the "Twelve" emulsion and a sterile emulsion. The net result of this method of preparing the recombination mixtures (illustrated diagrammatically in Figure 1) was that the "Four" and "Sixteen" recombinations contained equivalent amounts of the recombination of the 4 apparently significant organisms; and the "Twelve" and "Sixteen" recombinations contained equivalent amounts of "Twelve" -- the fraction presumed to be non-pathogenic. In addition, the 3 recombination

* See footnote on page D-3.

mixtures contained equivalent amounts of blood agar and broth.

Three groups of 5 guinea pigs matched for weight (300 to 360 gms.) and sex were inoculated with the recombination mixtures, each animal receiving 1 ml. of the appropriate emulsion subcutaneously in the groin. The animals were randomized in the cages and their positions changed daily. Their condition was recorded daily by one of us (JBM) who did not know to which group any of the animals belonged. Where possible, exudate was aspirated from appropriate lesions on or before the 7th day and passed to fresh animals in a dosage of 0.1 ml. This procedure was repeated through a 3rd passage as shown in Table II. The identity of the groups to which the animals belonged remained anonymous to the examiner to this point.

Results: Typical and indistinguishable abscesses developed in all 5 animals inoculated with "Sixteen" (total recombination (Table II)). The abscesses were well walled-off and contained abundant thin, creamy, foul-smelling exudate, yellow or grey-brown in colour. Animals inoculated with "Twelve" (presumed non-pathogenic) fraction in each case developed only small fibrotic nodules with no exudate. Passage to additional animals therefore was not possible within this group.

Typical abscesses developed in all 8 animals of the 2nd and 3rd passages in the "Sixteen" group and in 5 of 8 guinea pigs in the "Four" group. There was an apparent decrease in infectivity of the exudate used for 3rd passage inoculation in "Four" group (only 1 typical abscess in 4 animals) but typical abscesses and also spreading lesions occurred in 4 subsequent passages of the "Four" group (Table I).

All exudates were examined by dark field. In the case of exudates derived from "Sixteen" the appearance was typical of fusospirochetal material; those from "Four" disclosed only rods, most of which were non-motile. Exudate from 10 animals in various passages of the "Four" recombination was streaked on blood agar and incubated aerobically and anaerobically for 7 days. In every instance the original 4 organisms (strains K110, K92, JR3 and K80) were recovered without evidence of contamination.

Discussion: The distinction between infectivity and transmissibility on the basis of capacity to produce infection in the first as opposed to subsequent passages is in a sense artificial. The first passage was suitable for measuring infectivity. Under the conditions of these experiments, transmissibility was

a measure of the capacity of the infective agents to persist in the exudate, i.e., a measure of the retention of infectivity. Thus the results in Table I suggest that in certain instances infectivity differed between first and subsequent passages. Discounting the instances in which individual experiments appeared to be in conflict with the overall results (groups G, M and O), it can be seen that omission of either JR3 or K80 resulted in a loss of infectivity (transmissibility) beyond the first passage whereas in the first passage typical infection did occur in the absence of either of these organisms. The role of strain JR3 as noted is apparently directly related to the maintenance of strain K110. The role of strain K80 in maintaining infectivity has not been shown.

The minimum combination of organisms capable of eliciting typical "fusospirochetal" responses in the guinea pigs as shown in these studies is not necessarily the only combination which would be effective. For example, it is possible that one or other of the 4 organisms found to be significant could be replaced by one or other of the several strains of cocci or by the spirochetes. That interchange of certain of these organisms might be possible is suggested by the observation that several of the organisms produce the 'growth factor' required for strain K110. Strain JR3 might therefore be replaceable by one of these.

The striking finding in these experiments is that infection having all the attributes of fusospirochetal infection can be produced with a combination of organisms devoid of both spirochetes and fusiforms. Likewise, cocci and vibrios were non-essential, in this instance, although representatives of all of the above 4 types were reported necessary by both Smith (1930) and Proske and Sayers (1934). On the other hand, Kritchewski and Seguin (1920) reported that typical infections in the guinea pig resulted from combinations of spirochetes, fusiforms and either a greening streptococcus or a white staphylococcus. The divergency of these results supports the view that these mixed infections may be non-specific in a bacteriologic sense. The uniformity of the lesions produced from a variety of sources does suggest however a biochemical specificity -- a need for a particular combination of metabolites, toxins or enzymes not produced by any one organism.

The final experiment offers convincing evidence that only a small fraction of the complex mixed flora is required to produce typical infections and that the majority of the organisms

play no essential role. The findings in the first passage in this experiment (Table II) are the only ones which are strictly comparable. The inoculum in each of the 3 groups (pathogens versus total, and total versus non-pathogens) were made equivalent in respect to bacterial content and the findings are clear cut. Infection developed in every animal where the pathogenic quartette was present in the inoculum and in none of 5 animals where only the remaining 12 organisms were injected. Direct comparisons beyond the first passage are hazardous because no data are yet available concerning fluctuations in bacterial count from exudate to exudate. There is therefore no assurance that dosage in terms of numbers of viable organisms was comparable beyond the first passage. Nevertheless, the results demonstrate further that transmissible infection indistinguishable from unpurified fusospirochetal infection can be produced with a combination of only 4 of the 17 organisms composing the total flora of this particular exudate.

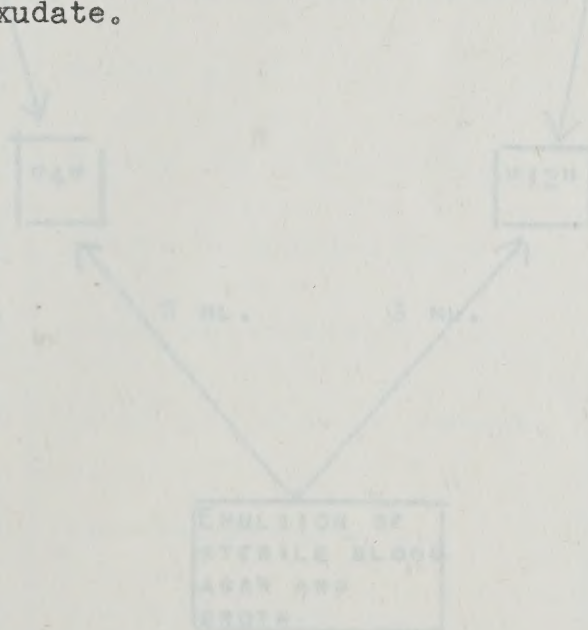


FIGURE 1. DIAGRAMMATIC REPRESENTATION OF COMPOSITION OF RECOMBINATION MIXTURES.

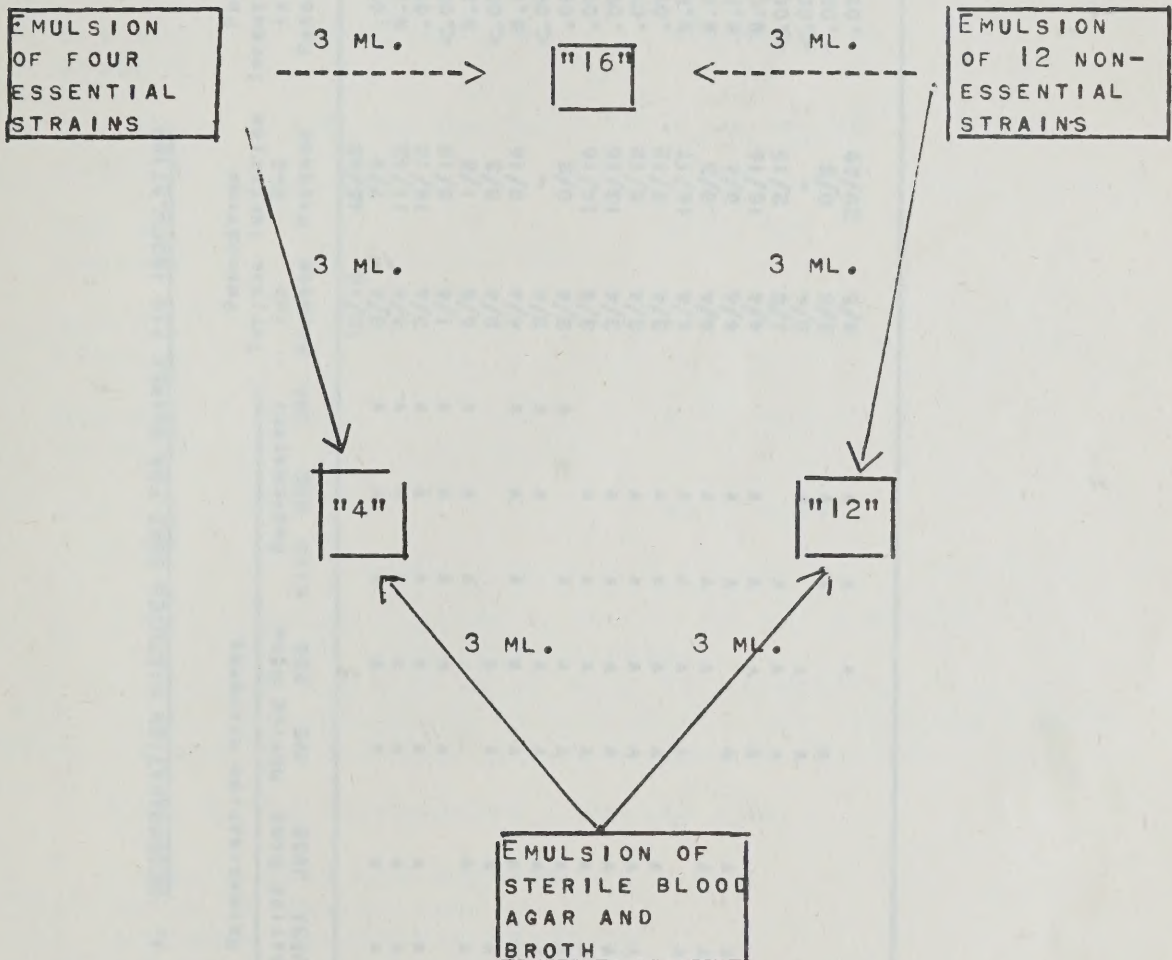


TABLE 1. RECOMBINATION MIXTURES USED FOR GUINEA PIG INOCULATION

GROUP	COMPOSITION OF RECOMBINATION MIXTURES										PROPORTION TYPICAL INFECTION		PROBABILITY VALUES	
	SPIRO- CHETES	COCCI	FUSIFORM JF3	GRAM JR3	POSITIVE JB3A	RODS JB3B	MOTILE JV5	RODS K80	K110	K92	BACTEROIDES JR6	1st PASSAGE	2-5 PASSAGE	2-5 PASSAGE
CONTROL														
A	V	V	V	V	V	V	V	V	V	V	V	12/12	46/48	.03
B	V	V	V	V	V	V	V	V	V	V	V	3/4	7/9	.55
C	V	V	V	V	V	V	V	V	V	V	V	4/4	11/12	.23
D	V	V	V	V	V	V	V	V	V	V	V	3/4	14/16	.07
E	V	V	V	V	V	V	V	V	V	V	V	1/4	8/15	<.001
F	V	V	V	V	V	V	V	V	V	V	V	6/6	1/8	<.001
G	V	V	V	V	V	V	V	V	V	V	V	0/4	0/3	<.001
H	V	V	V	V	V	V	V	V	V	V	V	4/4	9/16	<.001
I	V	V	V	V	V	V	V	V	V	V	V	0/4	-	-
J	V	V	V	V	V	V	V	V	V	V	V	2/4	0/2	<.001
K	V	V	V	V	V	V	V	V	V	V	V	3/4	14/16	.23
L	V	V	V	V	V	V	V	V	V	V	V	3/4	13/16	.05
M	V	V	V	V	V	V	V	V	V	V	V	3/4	5/12	<.001
N	V	V	V	V	V	V	V	V	V	V	V	3/4	8/12	<.001
O	V	V	V	V	V	V	V	V	V	V	V	4/4	16/17	.73
P	V	V	V	V	V	V	V	V	V	V	V	4/4	0/3	<.001
Q	V	V	V	V	V	V	V	V	V	V	V	0/4	0/4	<.001
R	V	V	V	V	V	V	V	V	V	V	V	4/4	16/16	.39
S	V	V	V	V	V	V	V	V	V	V	V	1/3	2/15	<.001
U	V	V	V	V	V	V	V	V	V	V	V	0/4	-	-
V	V	V	V	V	V	V	V	V	V	V	V	2/5	0/5	<.001
W	V	V	V	V	V	V	V	V	V	V	V	4/5	25/28	.23

N.D. = NO DEVIATION FROM EXPECTED.

TABLE II. PATHOGENICITY OF DIFFERENT RECOMBINATION MIXTURES IN GUINEA PIGS

PASSAGE	INOCULUM	"16" GROUP	"4" GROUP	"12" GROUP
1	1 ML. CULT. MASH	*+, *+, +, +, ++	++, ++, +, +, +	±, ±, ±, ±, ±
2	0.1 ML. EXUDATE	+, +, ++*, ++	++, +, +, +	
3	0.1 ML. EXUDATE	++, +, ++, ++	±, ±, ±, +*	
4	0.3 ML. EXUDATE		++*, +++	
5	0.3 ML. EXUDATE		++*, +++	
6	0.3 ML. EXUDATE		++*, ++	
7	0.1 ML. EXUDATE		+++ , ++++	

KEY: ± SMALL NODULAR LESION; LITTLE OR NO EXUDATE.
 + LESS THAN 1" LOCALIZED ABSCESS.
 ++ LOCALIZED ABSCESS 1" OR MORE IN DIAMETER.
 +++ LOCALIZED ABSCESS WITH EXTENSION; NOT FATAL IN 7 DAYS.
 ++++ SPREADING INFECTION FATAL IN 7 DAYS OR LESS.

EACH SYMBOL REPRESENTS 1 GUINEA PIG.

* EXUDATE USED FOR PASSAGE
 "16" TOTAL RECOMBINATION
 "4" PRESUMED PATHOGENIC FRACTION
 "12" PRESUMED NON-PATHOGENIC FRACTION

APPENDIX E

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: The cultivation and characteristics of Bacteroides nigrescens.

Grantee: Dr. J. B. Macdonald,
University of Toronto.

Project No.: DR-50 Amount of Grant: \$400.00

SUMMARY OF WORK

Examination of various media for production of the growth factor required for Bacteroides nigrescens indicated that peptone water was the simplest satisfactory medium. The synthetic medium previously described was not satisfactory. Growth of Bacteroides nigrescens is supported well in Difco thioglycollate broth plus filtrate of staphylococcus growth in peptone water.

Three strains of Bacteroides nigrescens produced indol and it is hoped that an assay may be developed based on utilization of tryptophane.

A micronutritional assay method based on the length of a growth column in heavily seeded agar tubes was unsatisfactory because of non-viability of the washed cells used for seeding.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: The Cultivation and Characteristics of
Bacteroides Nigrescens

Grantee: Dr. J. B. Macdonald
University of Toronto

Project No.: DR-50

Amount of Grant: \$400.00

PROGRESS REPORTIntroduction

Efforts have been directed primarily at developing a satisfactory method of assay for the essential growth factor for certain strains of Bacteroides nigrescens found in filtrates of broth culture of Micrococcus aureus. The initial observation of the relationship between these organisms was made on blood agar plates streaked with Bacteroides nigrescens and cross-streaked with Micrococcus aureus. A band of pigmented growth of variable width parallel to the staphylococcus streak resulted after 10 to 14 days anaerobic incubation. Although the width of this band is probably a function of the amount of growth factor elaborated use of this method for an assay is not practical because of the long incubation period required.

1. Medium for production of growth factor.

A previously described synthetic medium was prepared for cultivation of the staphylococci (see minutes 19th Meeting). This medium was found satisfactory for support of growth of Micrococcus aureus but no growth factor for Bacteroides nigrescens was produced.

Tests were then made to determine the simplest medium in which the growth factor was elaborated. Media tested included 5% of vitamin-free casein hydrolysate, 5% solution of acid hydrolyzed casein, 5% Bacto-casitone (an enzymatic hydrolysate of casein), 2% peptone water (Difco). To each of the above was added 2.5% $\text{FeSO}_4(\text{NH}_4)_2 \text{SO}_4$, 1% MgSO_4 , 1% NaNO_3 . Micrococcus aureus was inoculated into each of the media and incubated for periods of 12, 24, 36 and 48 hours. The cultures were filtered and the filtrate added to a variety of fluid media to a final concentration of 20%. Both Seitz and sintered glass filtrates were tried with comparable results. The media plus added filtrate were inoculated with Bacteroides nigrescens with a loop from surface growth on blood agar and incubated for 2 weeks. Control media without filtrates were also inoculated.

The results are shown in Table 1. It can be seen that growth factor for all 3 strains tested was produced only in peptone water and further that its presence could be demonstrated regularly only in Difco thioglycollate broth or BBL dextrose broth. These results were confirmed by several repetitions. Controls in no instance showed any evidence of growth.

While there are a number of possible explanations for the difference in behaviour of strain DR₂ compared to other 2 strains, it may be that the difference is based on requirements for the growth factor which differed quantitatively. It is thought that the effectiveness of the thioglycollate media is associated with a lower O-R potential.

The results indicate that 2% peptone water is a satisfactory simple medium for the production of the growth factor.

2. Biochemical Tests

In a search for a rapid method of assaying for the growth factor tests were made for metabolites which could possibly be used as indicators early in the growth cycle. Examinations were made for indol production, H₂S, acetylmethylcarbinol. Of these three, only indol was formed. It is not yet known how early indol is produced but this point is being studied. In the event of early production of indol an assay might be developed based on utilization of tryptophane.

3. Seeded Tubes

Shils, Horowitz and others (SCIENCE 115:517, 1952) described a micronutritional assay based on the length of the growth column in heavily seeded agar tubes overlaid with the test substance. The diffusion into the agar was a function of concentration and was disclosed by the length of the column. This method was tried although even if successful, it was likely to suffer from the disadvantage of a long incubation period.

Cells recovered from blood agar were washed and re-suspended in saline and used for seeding the tubes. Tests of the viability of the heavy suspension were made by the cross-streaking method on blood agar. Most of the cells were found to be non-viable as a result of the washing. Washing was tried also in distilled H₂O, and 1:10,000 Tween 80 or Tween 20 with the same results.

Table 1. Growth of 3 strains of *Bacteroides nigrescens* in various media

Strain	Medium					
	B236		Psafke		BBL Dextrose	
	K110	TH ₄ DR ₂	K110	TH ₄ DR ₂	K110	TH ₄ DR ₂
Casein hydrolysate	0	+	0	+	0	+
Vitamin-free						
Casamino acids	0	+	0	+	0	+
Bacto-casitone	0	+	0	+	0	+
Peptone	+	+	0	+	+	+

Legend:

0 = no growth

+ = growth

B236 = Difco thioglycollate
 Psafke = Veal heart infusion - ascitic fluid - kidney extract
 BBL = Baltimore Biological thioglycollate
 HI = Heart infusion

Filtrate

APPENDIX F

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: The amino acid composition of enamel protein using paper chromatography.

Grantee: Dr. G. Nikiforuk,
University of Toronto.

Project No.: DR-57

Amount of Grant: \$3,200.00

SUMMARY OF WORK

Preliminary work on this project has been proceeding along two general lines:

- 1) Preparation and purification of enamel powder
- 2) Overcoming technique problems associated with paper chromatography of enamel hydrolysates.

The flotation-centrifugation technique has been used in preparing enamel powder in which the dentin impurity has been reduced to 0.1 - 0.2 per cent "junction" particles. This material is to be used for amino acid analysis.

In the preliminary chromatography experiment it has been noted that the washing of the filter paper results in marked improvement in the chromatography of amino acids. A seven stage washing of the paper for a period of 52 days is described.

Apparatus has been assembled to permit a 'mapping' of known amino acids.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: The Amino Acid Composition of Enamel Protein Using Paper Chromatography.

Grantee: Dr. Gordon Nikiforuk
University of Toronto

Project No: DR-57

Amount of Grant: \$3,200.00

INTERIM REPORT

Attention in this project was first centered on the development of suitable techniques.

1. Preparation and Purification of Powdered Enamel

A. Preparation

Sound extracted teeth were stored in acetone and later dried at 100° C. for 6 hours. This permitted enamel to be chipped off readily. The exterior surfaces were lightly ground with a diamond stone to remove all visible pit and fissure material. The cleaned fragments were pulverized in a diamond mortar, after which metal particles were removed magnetically. It was found that the impurity in terms of dentin particles was greatest in the larger particle sizes. The powder that passed a 100-mesh was found most suitable for subsequent purification.

B. Purification of Enamel

Two techniques have been tried. (I) Flotation by use of liquid with graduated density. A tall glass cylinder is partly filled with one organic liquid (bromoform) and the remainder of the cylinder filled with acetone -- miscible with the first but lighter. The mixture is mixed gently using a 'spiral' wire stirring rod commencing at the junction of the two liquids and gradually extending along the cylinder length. This gives a mixture in which the density is graduated more or less uniformly from the lower to the higher value. The enamel powder is poured into the cylinder and separates according to the density of the components, enamel (2.89-3.00); dentin (2.05-2.35). This method was satisfactory for initial separations. However it was difficult to get the desired degree of purity because the heavier enamel particle appeared to carry some dentin impurities during settling. (II) Flotation-centrifugation technique. A bromoform-acetone mixture (density adjusted to 2.75) was used to separate enamel from dentin. 3 parts of the mixture to 1 part of enamel powder was used. The powdered enamel was stirred gently for 2 minutes and then centrifuged. This was

repeated from four to eight times until the dentin impurities were reduced to a point where 10 - 20 "junction" particles were observed in a count of 1,000. Enamel particles were differentiated from dentin on the basis of different refractive indices -- dentin (1.56) and enamel (1.60). A liquid with a refractive index of 1.59 is prepared by mixing 76 vol. % of α -chloronaphthalene and 18 vol. % of liquid petrolatum was found most suitable for viewing the powdered enamel.

Enamel with impurity of 1 - 2 % "junction particles" was prepared in sufficient quantities to permit preliminary analysis.

2. Preliminary technique problems associated with paper chromatography.

A. Washing of Filter Paper: Purification of the filter paper results in marked improvement in the chromatography of amino acids and peptides. The advantages vary but include frequently the formation of more circular spots, cleaner separation and more rapid 'running'. Washing of the paper involved seven stages and requires 52 days. Large numbers of paper (60) may be washed at once. The procedure used is essentially as developed by Hanes (1954) and is summarized as follows:

Stage	Extractant per block of 60 sheets	Volume	Duration	Suction
1	2N Acetic Acid	5 litres	3 days	Continuous
2	Water	7 "	7 "	Continuous
3	0.5N Lithium hydroxide	12 "	22 "	Intermittent
4	Water	10 "	9 "	Intermittent
5	0.1% (W/V) calcium acetate	4½ "	3 "	Intermittent
6	Water	8 "	5 "	Continuous
7	95% (v/v) Ethanol	6 "	4 "	Continuous

B. General apparatus common to most chromatography techniques has now been assembled and the preliminary work of preparing a chromatography map of known amino acids is now in progress.

The assistance of Professor C. S. Hanes from the Department of Biochemistry, University of Toronto, is most gratefully acknowledged.

Bibliography: (1) Hanes, C. S., Personal Communication, 1954.

(2) Manley, R. S. and Hodge, H. C., Jour. Dent. Res., 18:133, 1939.

APPENDIX G

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: An investigation of the mechanism of repair of the supporting structures of the teeth.

Grantee: Dr. Charles H. Best,
University of Toronto.

Project No.: DR-22

Amount of Grant: \$4,200.00

SUMMARY OF WORK

The earlier work in this investigation has been largely a basic study under experimental conditions of the mechanisms of the regenerative process involved in the reattachment of the soft tissues to the teeth and the rebuilding of the bone of the socket. While much had been learned from our earlier experiments, our knowledge of the regenerative process was still inadequate to meet the more complex conditions presented by epithelialized pockets, the type that occurs when the loss of supporting structure is produced by periodontal disease. Two main approaches were decided on; first to continue work on the as yet unknown factors in the regenerative process, and second, to attempt to provide in epithelialized pockets the conditions for inducing the desired growth of bone, connective tissue and cementum. In this report the work is presented under these two main headings:-

No. 1 - Basic Studies

(a) A study was planned and carried out for the purpose of determining whether or not replacement cells originating from the gingiva are suitable for regeneration purposes or if the replacement cells must originate from the alveolar process and periodontal membrane at the margins of the pocket. This study has been completed but the results are not conclusive. The method in general seems to be satisfactory but a further step in the procedure will probably result in more conclusive evidence. This study is being repeated with the necessary changes.

(b) One of the differences between the experimental conditions in study 2 where regeneration was successfully induced and the conditions in epithelialized pockets, is that in the former periosteum is present in the detached gingiva. This is not the case in epithelialized pockets. A study is being carried out to investigate the role of the periosteum in the

regeneration achieved in study 2. The main approach to this problem is by the use of polyethylene tubing to repair gaps in the fibula of dogs. The gaps are made by removing from the fibula a section of both bone and periosteum. Bone has been successfully grown through the tubing. This method appears to be a valuable research tool in studying bone regeneration. Dr. C. H. Best and Dr. I. McNab of the Department of Orthopedics are actively interested in this phase of the work.

(c) Dr. Best, Mr. Salter and Mr. Lawrence are conducting an investigation of hormonal factors affecting growth. I am collaborating with them by studying the histological changes induced by their experimental procedures in bone and connective tissues. For various reasons the supporting structures of the teeth provide an excellent index of osteogenic and connective tissue changes.

(d) In this department material is available for a study of the effect on the tissues of the periodontium of endocrine and other factors controlling metabolism. Advantage is being taken of this opportunity as time permits.

No. 2 - Regeneration from epithelialized pockets.

(a) As reported at the last meeting, reattachment and regeneration was successfully induced in epithelialized pockets in dogs. Additional cases have been completed since that time. In the case shown at the meeting where the healing period was short, the epithelium lining the pocket wall was still present in the reattached gingiva. Since then cases with a longer healing period have been completed. Fortunately it was found that with the longer healing period the epithelium in the gingiva disappears and is replaced by connective tissue.

(b) In study 3, (in press) a mechanism was found which produced a slow regeneration of supporting structure in periodontal pockets. Four cases have been completed where a partial regeneration of supporting structure was achieved by the surgical approach. The amount of bone regeneration was carefully measured by remaking a gingival flap. The flap was sutured into place and the animals have been kept for a fairly long period to determine if there is a further growth of bone by the mechanism described in study 3.

(c) Early changes in the surgical approach to regeneration have as yet not been studied. It is planned to proceed with a study of these early changes.

This year one paper reporting this work has been finished and another is in preparation. Reprints will be sent to the Committee as soon as they are available.

(Submitted by W.J. Linghorne)

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: In investigation of the mechanism of repair of the supporting structures of the teeth.

Grantee: Dr. Charles H. Best,
University of Toronto.

Project No.: DR-22

Amount of Grant: \$4,200.00

INTERIM REPORT

The earlier work in this investigation has been largely a basic study under experimental conditions of the mechanism of the regenerative process involved in the reattachment of the soft tissues to the teeth and the rebuilding of the bone of the socket. While much had been learned from our earlier experiments, our knowledge of the regenerative process was still inadequate to meet the more complex conditions presented by epithelialized pockets, the type that occurs when the loss of supporting structure is produced by periodontal disease. Two main approaches were decided on, first to continue work on the as yet unknown factors in the regenerative process and second, to attempt to provide the conditions in epithelialized pockets for inducing the desired growth of bone, connective tissue and cementum. In this report the work is presented under two main headings.

No. 1. Basic Studies

(a) A study was planned and carried out for the purpose of determining whether or not replacement cells originating from the gingiva are suitable for regenerative purposes or if the replacement cells must originate from the alveolar process and periodontal membrane at the margins of the pocket. This study has been completed but the results are not conclusive. The method in general seems to be satisfactory but a further step in the procedure will probably result in more conclusive evidence. This study is being repeated with the necessary changes.

(b) One of the differences between the experimental conditions in Study 2 where regeneration was successfully induced and the conditions in epithelialized pockets, is that in the former periosteum is present in the detached gingiva. This is not the case in epithelialized pockets. A study is being carried out to investigate the role of the periosteum in

the regeneration achieved in Study 2. The main approach to this problem is by the use of polyethylene tubing to repair gaps in the fibula of dogs. The gaps are made by removing a section of both bone and periosteum. Bone has been successfully grown through the tubing. This method appears to be a valuable research tool in studying bone regeneration. Dr. Best and Dr. McNab of the Department of Orthopedics are actively interested in this phase of the work.

(c) Dr. Best, Mr. Salter and Mr. Lawrence are conducting an investigation of hormonal factors affecting growth. I am collaborating with them and studying the histological changes induced by their experimental procedures in bone and connective tissue. For various reasons the supporting structures of the teeth provide an excellent index of some osteogenic and connective tissue changes.

Histological sections of the periodontal structures have been prepared from the following experimental groups.

- Group 1 - Normal rats
- Group 2 - Hypophysectomized rats
- Group 3 - Hypophysectomized rats with insulin
- Group 4 - Hypophysectomized rats with growth hormone

Comparable sections are now being prepared from more recent experiments. These groups are:

- Group 1 - 1 to 12 Hyped controls - no treatment
- Group 2 - 13 - 24 Hypophysectomized rats treated with growth hormone treated for 21 days
- Group 3 - 25 - 44 Hypophysectomized rats treated with insulin - 21 days
- Group 4 - 45 - 64 Hypophysectomized rats treated with insulin and growth hormone - 21 days.

(d) In this department material is available for a study of the effect on the tissues of the periodontium of endocrine and other factors controlling metabolism. As time permits advantage is being taken of this opportunity. Through the courtesy of Dr. R. E. Haist and Dr. M. Nishikawara, sections are being made of the following experimental groups of rats.

- Group 1 - 1 - 8 normal ad lib
- Group 2 - 9 - 36 hyped ad lib with their individual normal pair-fed controls
- Group 3 - 37 - 64 hyped treated with ACTH and their individual normal pair-fed controls
- Group 4 - 65 - 84 normal rats treated with ACTH and their normal pair-fed controls

No. 2. Regeneration from epithelialized pockets.

(a) As reported at the last meeting reattachment and regeneration was successfully induced in epithelialized pockets in dogs. Additional cases have been completed since that time. In the case shown at the meeting where the healing period was short, the epithelium lining the pocket wall was still present in the reattached gingiva. Since then cases with a longer healing period have been completed. Fortunately it was found that with the longer healing period the epithelium in the gingival disappeared and is replaced by connective tissue.

(b) In study 3 (in press), a mechanism was found which produced a slow regeneration of supporting structure in periodontal pockets. Four cases have been carried out where a partial regeneration of supporting structure was achieved by the surgical approach. The amount of bone regeneration was carefully measured by remaking a gingival flap. The flap was sutured into place and the animals are kept for a fairly long period to determine if there is a further growth of bone by the mechanism described in study 3.

(c) Early changes in the surgical approach to regeneration have as yet not been studied. These changes are now being studied.

This year one paper reporting this work has been finished and another is in preparation. Reprints will be sent to the Committee as soon as they are available.

One of the serious obstacles in this project has been the difficulty of preparing histological paraffin section of dog's teeth and supporting structures. Celloidin sections are the standard method for preparing sections of these tissues. However, the thickness of celloidin sections made them unsuitable for the cytological studies in the work. Fortunately Dr. H. Hunter and Mr. G. Pudymaitis of the Research Division of the Faculty of Dentistry have developed a method for making excellent paraffin sections of these tissues. Recently splendid equipment for the preparation and cutting of bone and tooth sections has been provided for this project by our department. Mrs. J. Ashdown, my assistant, has been trained in the new techniques. It is hoped that soon a serious bottleneck will be overcome.

(Submitted by W. J. Linghorne)

APPENDIX H

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject : Chemical Mechanisms in Dental Caries

Grantee : J. J. Rae
University of Toronto

Project No.: D.R. 1

Amount of Grant: \$1,080.00

SUMMARY OF WORK

Ammonia Production

In the presence of urea and glucose saliva produces larger quantities of ammonia than when urea alone is present. Wide variations in the ratios of urea and glucose had little effect on ammonia production. In all cases, the pH was maintained at a higher level than where glucose alone was used.

Samples of chocolate biscuit-coating supplied by Consumers' Research Laboratories containing one, two and ten per cent urea were mixed with saliva and incubated for 24 hours. A direct relationship was observed between urea content and ammonia production and between urea content and pH. Sucrose could be substituted for glucose in ammonia production experiments with analogous results. Dulcin (para-phenetyl-urea), an artificial sweetener that does not have the disagreeable bitter taste of urea, did not liberate ammonia when acted on by salivary urease. A final concentration of 0.6% urea was found to maintain the pH at 7.1 after 26 hours incubation of a urea-chocolate mixture with saliva.

Gardol (sodium lauryl sarcosinate) showed only a slight inhibition of acid production when added to an incubation mixture of saliva and glucose.

A colorimetric method for the determination of urea in saliva was found to be necessary if this work is to be continued and a modified Ormsby-Barker^{1,2} method seems to meet most of our requirements.

1. A.A. Ormsby, J.B.C. 146: 595, 1942.
2. S.B. Barker, J.B.C. 152: 453, 1944.

APPENDIX I

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Investigation of methods for destroying the organic matter in teeth, bones and other materials prior to determination of fluorine content.

Grantee: Dr. O.J. Walker,
University of Alberta.

Project No.: DR-28

Amount of Grant: Nil

SUMMARY OF WORK

No further report has been carried out on this project since the last report was made. We have had the lumetron photoelectric colorimeter reconditioned and repaired on funds provided by the Committee on Dental Research.

During the coming winter Miss Hinda Doz who worked on the project last summer will be able to spend part of her time on the continuation of the problem. A report will be made later on her results.

Additional work has been carried out on the analysis of flour. Flour has been added to Newfoundland flour since 1947 and the flour was found to contain 2.8 p.p.m. fluorine. The teeth were from children who had consumed this flour over a 10 year period. The results when compared with Brantford teeth showed both the deciduous and permanent dentin content of fluorine to be higher than the Brantford teeth with 3 years exposure; on the other hand the enamel fluoride content in both cases was lower than that of the Brantford teeth although higher than that of the control teeth from Sarnia. The results are also presented graphically in two figures.

A study of nutrition records from 1932 of the Newfoundland children from whom the teeth had been extracted when compared with the corresponding dental caries record showed an indirect relationship between high nutrition scores and high caries scores.

APPENDIX J

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Fluoride content of dentin and enamel of teeth
from Sarnia, Brantford and Stratford.

Grantee: Dr. M. Doreen Smith,
University of Toronto.

Project No.: DR-13

Amount of Grant: \$1,625.00

SUMMARY OF WORK

Additional teeth from Brantford and Sarnia have been analyzed for fluorine according to the procedure reported in March 1954. The results are reported in three tables and it can be seen that they follow the pattern of those reported previously. That is, both dentin and enamel fluoride content of teeth with 7 to 8 years exposure to fluoridated water is higher than the Sarnia controls and higher than that found in teeth with $3\frac{1}{2}$ years exposure.

Results are also given of analyses of deciduous and permanent teeth from Newfoundland children (2 - 17 years of age). Bonemeal has been added to Newfoundland flour since 1947 and the flour was found to contain 2.8 p.p.m. fluorine. The teeth were from children who had consumed this flour over a $3\frac{1}{2}$ year period. The results when compared with Brantford teeth showed both the deciduous and permanent dentin content of fluorine to be higher than the Brantford teeth with 3 years exposure; on the other hand the enamel fluoride content in both cases was lower than that of the Brantford teeth although higher than that of the control teeth from Sarnia. The results are also presented graphically in two figures.

A study of nutrition records from 282 of the Newfoundland children from whom the teeth had been extracted when compared with the corresponding dental caries record showed an indirect relationship between high nutrition scores and high caries scores.

APPENDIX J

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Fluoride content of dentin and enamel of teeth
from Sarnia, Brantford and Stratford.

Grantee: Dr. M. Doreen Smith,
University of Toronto.

Project No.: DR-13

Amount of Grant: \$1,625.00

INTERIM REPORT

This interim report is primarily on a continuation of a study of the effect of water fluoridation on the fluorine content of enamel and dentin of deciduous and permanent teeth from Brantford (1 p.p.m. artificial water fluoridation) using teeth from Sarnia (no fluorine in water) and Stratford (1 p.p.m. naturally in water) as controls.

The separation of enamel and dentin of the teeth and the method of analysis for fluorine is the same as in the March, 1954 report on this project. Eighteen deciduous and twenty permanent teeth from Brantford have been received and analyzed and the results reported in Tables I and II. Two additional teeth from Sarnia were also received and analyzed and are included in Table II. Another twenty teeth from Brantford are ready for analysis.

Miss Tung Yu Lin, who made the above analyses has also fractionated and analyzed a number of teeth from Newfoundland which were extracted by Dr. Pownall in 1951. These teeth were from children who consumed large amounts of bread and other carbohydrate foods (about 12 oz. of flour per capita per day). Bonemeal had been added to the flour since November 1947 and the flour was found on analysis by Miss Lin to contain 2.8 p.p.m. fluorine. Some of the children consumed tea which would contribute fluorine in a more soluble form to their diet and fish which is also high in fluorine.

Since the fluorine added through bonemeal to the Newfoundland diet is in a relatively insoluble form whereas the fluorine ingested by the Brantford children is in a soluble form, it is thought to be of some interest to compare these results. Unfortunately no teeth were obtained before the addition of bonemeal to the flour, but it is hoped through

the efforts of Dr. H. K. Brown that more teeth will be sent in so analyses can be made after a longer exposure to the bonemeal enriched flour.

Table III shows the analytical figures for fluoride content of pooled samples of deciduous and permanent teeth respectively. These teeth were separated and classified by fourth year dental students at the University of Toronto. Carious and non-carious teeth were analyzed separately. The results showed no relationship between the fluoride content and the type of teeth, and there was no significant difference in the fluorine content of carious and non-carious teeth. The mean fluorine content of deciduous teeth is lower than that of permanent teeth with regard to both dentin and enamel. This is in agreement with all the results of Brantford teeth.

Table IV presents the fluoride means of deciduous teeth from Newfoundland which have had approximately 3½ years exposure to bonemeal enriched flour, as well as the means of teeth with 3 years exposure and with approximately 8 years exposure to Brantford fluoridated water.

Table V presents similar results for permanent teeth except that the means for children under and over 8 years of age are given separately for the 8 year exposure group. The above data is also presented graphically for the deciduous teeth in Figure I and for the permanent teeth in Figure II.

On comparison of the Newfoundland deciduous teeth with those of Brantford, the dentin content of fluorine is found to be higher, whereas that of the enamel is lower. The balance studies of Ham and Smith * show some fluorine may be absorbed and retained when ingested in the form occurring in bonemeal. It is possible that some of this fluorine is accumulating in the dentin. Without controls on Newfoundland teeth this conclusion cannot be definitely made, but it is hoped when teeth of 7 to 10 years exposure are examined more can be learned. The lower figure for the enamel of the teeth exposed to fluorine in the more insoluble form also provides interesting material for speculation but again conclusions are not warranted.

The comparison of results of the permanent teeth give a somewhat similar picture. On Figure I it can be seen that the enamel fluoride content is slightly higher than those from the control area of Sarnia, but the dentin fluoride

* Reprints sent to the Committee, August, 1954.

content is over twice as high. In Newfoundland the incidence of dental caries is notably high. Dr. Pownall made careful records of the dental health of each child and Mrs. Pownall obtained nutrition records from the mother when possible. Through the courtesy of Dr. Grainger these records from 282 children were made available to us.

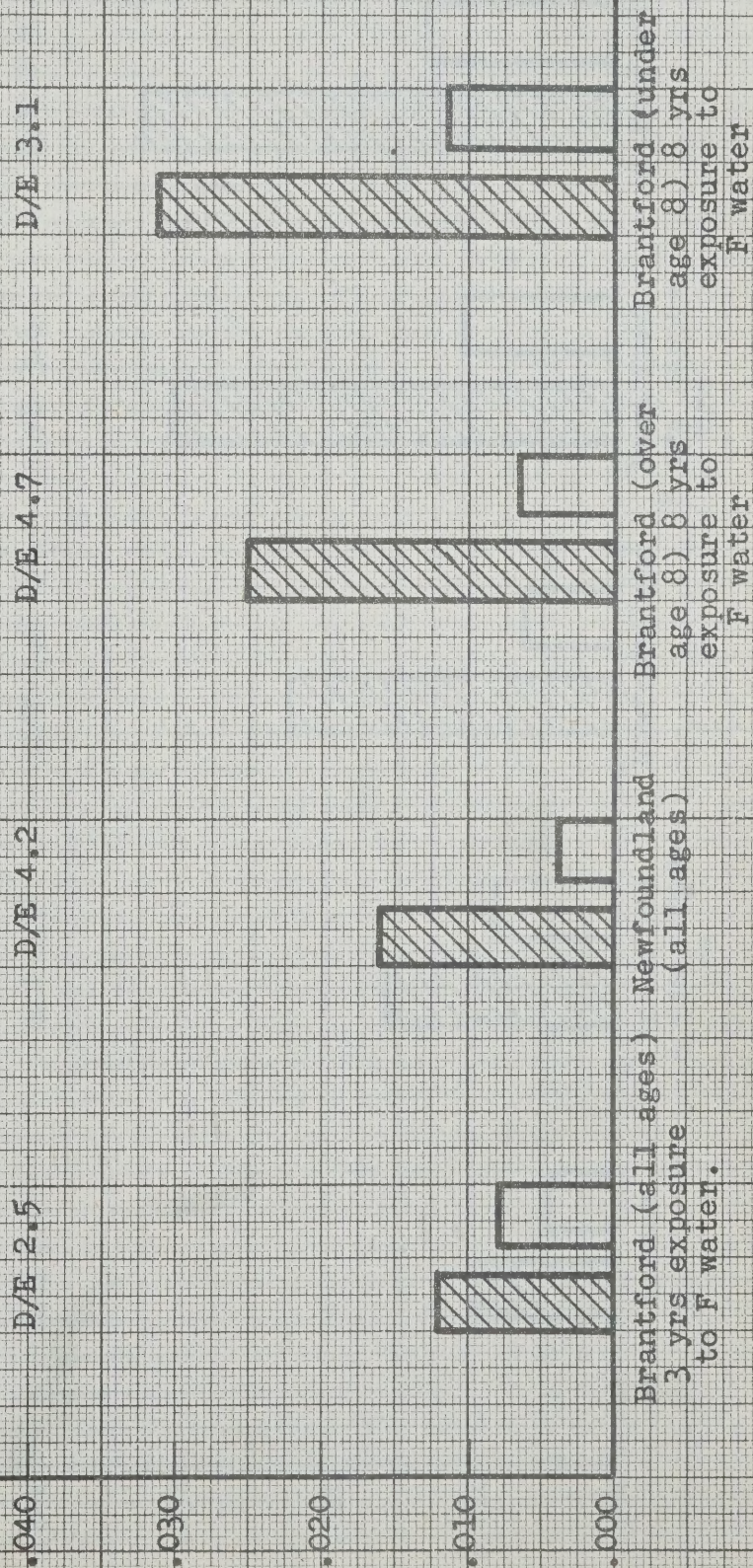
In a separate study made by Miss Lin the data on each card of the dental health and of the nutrition record were coded and scored. An indirect relationship was found between the high nutrition scores and high caries scores (d.m.f.) in the age groups of 2 to 6 and 9 to 17 years. Irregularities in the 6 to 8 year olds were probably due to the high number of missing teeth or the good condition of newly erupted permanent teeth.

GRAPH I

PERCENTAGE OF FLUORINE IN DENTIN AND ENAMEL OF DECIDUOUS TEETH



J-4



GRAPH II

PERCENTAGE OF FLUORINE IN DENTIN AND ENAMEL OF PERMANENT TEETH



J-5

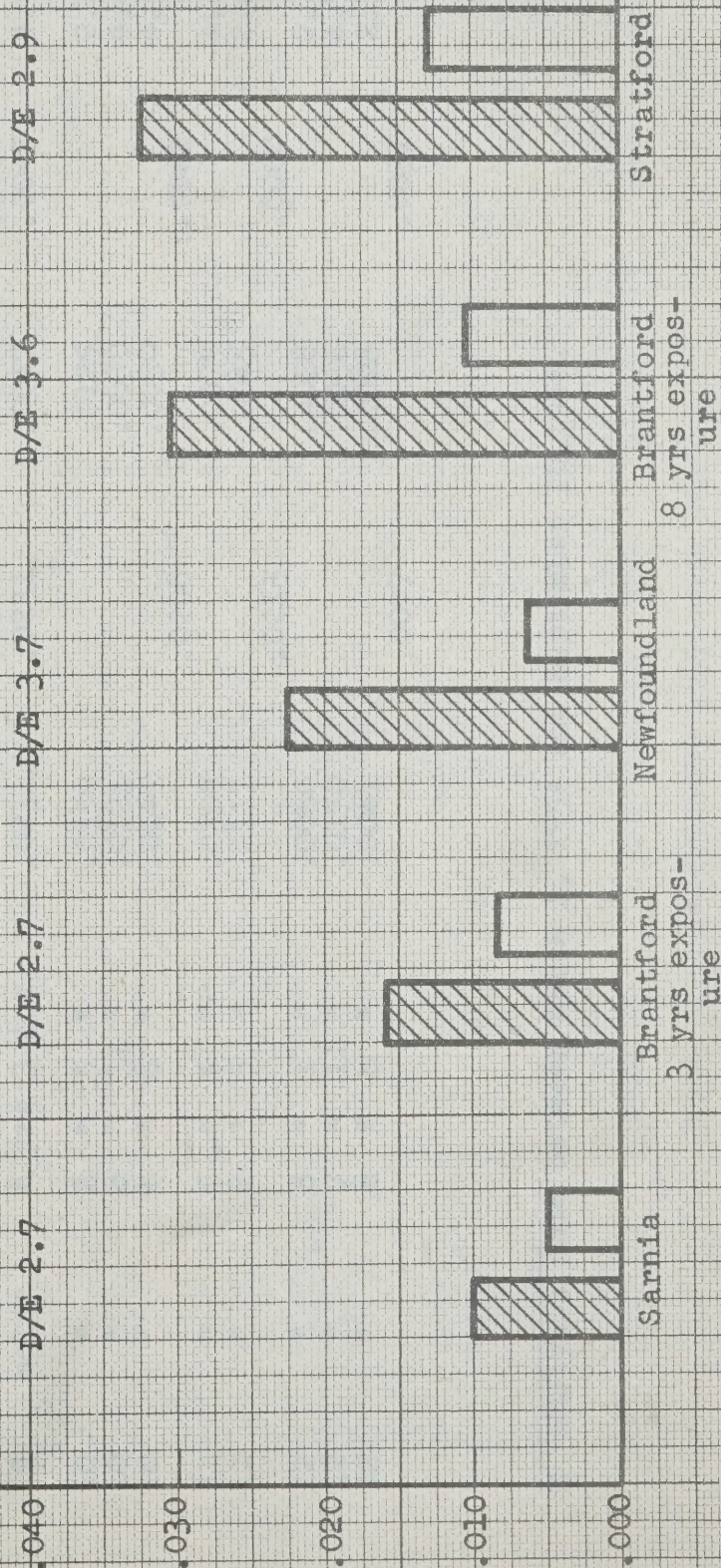


Table I

BRANTFORD DECIDUOUS TEETH *

Age	Type of tooth	Length of exposure	% Fluorine in dentin	Age mean	% Fluorine in enamel	Age mean	D/E
5	molar	8 yrs 4 mos	.0406		.0250		1.62
5	"	" 8 "	.0135		.0033		4.09
5	"	" 9 "	.0248		.0048		5.16
5	"	" 9 "	.0208		.0042		4.95
7	"	8 yrs 2 mos	.0209	.0249	.0057	.0093	3.66
7	"	7 yrs 10 mos	.0376		.0100		3.76
7	incisor	8 yrs 1 mo	.0423	.0336	.0182	.0113	2.32
8	molar	8 yrs	.0270		.0059		4.57
8	cuspid	8 yrs 11 mos	.0209		.0069	.0064	3.03
9	molar	8 yrs 3 mos	.0231	.0240 .0231	.0047	.0047	4.91
10	"	8 " 5 "	.0350		.0125		2.80
10	"	8 " 11 "	.0121		.0030		4.03
10	"	8 " 8 "	.0144		.0030		4.80
11	"	8 " 5 "	.0309	.0208	.0075	.0062	4.12
11	"	8 " 5 "	.0117		.0045		2.60
12	"	8 " 1 "	.0060	.0213	.0028	.0060	2.14
12	"	7 " 11 "	.0170		.0036		4.72
12	"	8 " 8 "	.0061	.0097	.0028	.0031	2.18

* Analysis made between March 1, 1954 and September 10, 1954.

Table II - BRANTFORD PERMANENT TEETH *

Age	Type of tooth	Length of exposure	% Fluorine in dentin	Age mean	% Fluorine in enamel	Age mean	D/E
7	incisor	8 yrs 3 mos	.0394	.0394	.0200	.0200	1.97
9	molar	8 " 11 "	.0110		.0040	.0200	2.75
9	bicuspid	8 " 9 "	.0282		.0112		2.52
9	"	8 " 8 "	.0226		.0085		2.66
10	molar	7 " 11 "	.0287	.0206	.0044	.0079	6.52
10	bicuspid	8 " 11 "	.0113		.0051		2.22
10	"	8 " 8 "	.0218		.0080		2.72
10	"	8 " 1 "	.0132		.0063		2.09
10	"	8 " 1 "	.0130		.0060		2.16
10	"	8 " 9 "	.0165		.0076		2.17
11	molar	8 " 2 "	.0123	.0174	.0038	.0062	3.24
11	bicuspid	8 " 11 "	.0155		.0068	.0053	3.28
12	"	8 " 11 "	.0220	.0139	.0058		3.79
12	"	8 " 10 "	.0279		.0070	.0064	3.98
13	molar	8 " "	.0225	.0250	.0072		3.12
13	"	7 " 11 "	.0236		.0045		5.24
13	bicuspid	8 " 9 "	.0092		.0028		3.29
13	"	8 " 8 "	.0128		.0023		5.56
14	molar	8 " 4 "	.0240	.0170	.0037	.0042	6.48
15	"	8 " "	.0201	.0240	.0050	.0037	4.02
15	"	8 " "		.0201		.0050	

* Analysis made between March 1, 1954 and September 10, 1954.

SARNIA PERMANENT TEETH

11	molar	.0059	.0025	2.36
16	"	.0065	--	--

TABLE III

% FLUORINE IN CARIOUS TEETH FROM NEWFOUNDLAND

Pooled Deciduous Teeth

Type	% of Fluorine in Dentin	% Fluorine in Enamel		D/E
		Type mean	Type mean	
Incisor	.0219			3.91
	.0141			3.28
		.0180	.0050	
Cuspid	.0205			3.36
	.0155			6.74
		.0180	.0042	
1st Molar	.0231			3.85
	.0139			4.96
		.0185	.0044	
2nd Molar	.0101			3.16
	.0124			4.96
		.0113	.0029	
Mean	.0164		.0041	4.25

Pooled Permanent Teeth

Incisor	.0160			2.29
	.0421			7.25
	.0172			2.07
	.0078			2.44
		.0208	.0061	
Cuspid	.0432			4.00
	.0486			4.86
	.0142			3.08
		.0353	.0085	
Bicuspid	.0281			3.74
	.0141			2.42
2nd Bicuspid	.0146			2.75
		.0189	.0059	
1st Molar	.0170			3.95
	.0176			2.00
	.0146			4.29
		.0164	.0055	
2nd Molar	.0112			2.95
	.0258			3.69
	.0316			7.18
	.0148			3.22
		.0209	.0050	
Mean	.0223		.0061	3.66

Percent Fluorine in Non-Carious Teeth

	% Fluorine in dentin	% Fluorine in enamel	D/E
Cuspid	.0317	.0077	4.12
	.0106	.0038	2.79
	.0106	.0038	2.79
Mean	.0176	.0051	3.23

Fluorine content in the white wheat flour of Newfoundland: 2.8 p.p.m.
fluorine (flour in ordinary state).

TABLE IV

PERCENTAGE OF FLUORINE IN DECIDUOUS TEETH

	% Fluorine in Dentin	% Fluorine in Enamel	D/E
Three years exposure to Brantford fluoridated water (all ages)	.0126	.0082	2.5
Newfoundland (all ages)	.0164	.0041	4.2
Eight years exposure to Brantford fluoridated water <u>Over age 8</u>	.0254	.0069	4.7
<u>Under age 8</u>	.0315	.0119	3.1

TABLE V

PERCENTAGE OF FLUORINE IN CHILDRENS' PERMANENT TEETH

	Dentin	Enamel	D/E
Sarnia	.0104	.0048	2.7
Brantford Fluoridated Water	3 years exposure .0163	.0084	2.7
	8 years exposure .0304	.0103	3.6
Newfoundland	.0223	.0061	3.7
Stratford	.0324	.0128	2.9

APPENDIX K

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Development of a rapid method for grinding thin sections of plastic-embedded teeth.

Grantee: B. N. Kropp,
Queen's University.

Project No.: DR-54 Amount of Grant: \$650.00

SUMMARY OF WORK

The last report dealt with a method of preparing teeth and bone for plastic embedding, and with the process of embedding.

The past 6 months were taken mainly with the design and construction of an instrument for cutting sections of plastic-embedded teeth at predetermined thicknesses, and also for making the instrument flexible enough to permit cutting serial sections of plastic-embedded teeth and bone at a single operation.

Both of these objectives have been achieved. An instrument is nearing completion that successfully cuts single sections as thin as 0.5 mm. or serial sections (in a single cutting operation) as thin as 1 mm. This has involved the design and construction of a suitable specimen holder, a device for advancing the specimen a measured distance, the experimental determination of suitable cutting tools, the determination of optimum speed of operation of the instrument, a device for carrying off dust from the bearing surfaces and the work.

Thickness Control

A micrometer head is mounted on the base plate of the specimen holder, and is used for advancing the specimen the required amount when cutting single consecutive sections.

Specimen Holder

The device mentioned on page 3 of my March, 1954 report has been completed. The base of the plastic cylinder holding the specimen to be cut is attached to the base of a $1\frac{1}{4}$ " clear plastic cylinder of any convenient length (but not less than 4"), by means of a thermoplastic cement or other plastic adhesive. The assembly is then mounted horizontally in such a way that the clear plastic $1\frac{1}{4}$ " cylinder is held in a brass sleeve, secured by knurled-head screws, and the portion holding the specimen projects in front of the saws.

Cutting

A screw device, controlled by a handle in front of the operator, advances the specimen towards the saws. The specimens are cut dry. It was found that if the speed of the saws is not too great, not over ca 3000 rpm, the danger of burning the tissue or melting the plastic is negligible. However, one precaution that must be observed is that the saws shall not have more than 120 teeth. Above that number the teeth will clog with sticky plastic dust and interfere with cutting.

Dust is carried away from the work and from the bearing surfaces of the instrument by means of a vacuum. In operation the saws and the work are covered by a protective transparent plastic hood.

The elimination of the necessity for a coolant has helped to simplify the design of this instrument. The major work involved in the design and construction of this instrument is almost completed. Additional work will have to be done on a method of grinding and polishing the 1 mm thick plastic-embedded specimens obtained by the serial cutting operation.

APPENDIX L

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements.

Grantee: Dr. C. P. Leblond (with Miss Y. Kumamoto), McGill University.

Project No.: DR-23

Amount of Grant: \$3,500.00

SUMMARY OF WORK

Studies on the mineralization of dentin utilizing radioactive calcium or phosphorus indicated that these elements were incorporated into the stable mineral salts of dentin in the layer of new dentinal matrix adjacent to predentin (Article 1).

To understand what happens at the predentino-dentinal junction, histochemical techniques were used particularly metachromasia with toluidine blue dye, and the periodic acid-Schiff reaction. These techniques suggested that the dentin, but not predentin, contained acid mucopolysaccharides (which are metachromatic) and neutral carbohydrates (stained by the periodic acid-Schiff technique).

The problem is to find out whether or not the addition of carbohydrates to the organic matrix of predentin enables it to become dentinal matrix, capable of retaining mineral salts and thus becoming the hard structure that it is in the fully grown teeth. Before attempting to answer this question, it was necessary to carry out chemical investigations to confirm the histochemical results. The data obtained this year confirmed that 2 types of carbohydrates exist in dentin: (1) the acid mucopolysaccharide, already investigated by Hess; and (2) a new type of carbohydrate protein complex, containing galactose, mannose, and fucose.

Several other lines of investigation were continued. Two articles were completed and submitted for publication.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements.

Grantee: Dr. C. P. Leblond (with Miss Y. Kumamoto),
McGill University.

Project No.: DR-23 Amount of Grant: \$3,500.00

INTERIM REPORTI. Carbohydrates of human dentin

To examine the nature of the carbohydrates of dentin, a large amount of material was necessary. It was, therefore, decided to use human teeth which could be obtained from a local clinic in large amounts.

Materials and Methods

Assorted human teeth were decalcified in 0.2% hydrochloric acid solution until the cementum and part of the enamel could be scraped off. Those parts of the crown which still were covered by enamel or any caries contaminated areas were then removed by a fine hand saw. The teeth, which now consisted of dentin and pulp, were hammered gently to open the pulp cavity and the pulp tissue removed whenever visible. The sample, which now consisted of dentin only, was encased in a wire mesh and hammered into small pieces. The purpose of the wire mesh was to keep the broken chips of teeth from flying away. Bits of pulverized mesh which became mixed with the dentin were removed by passing a magnet through the dentin sample.

The pulverized dentin was put into a Florence flask and further decalcified by 0.2% hydrochloric acid solution. To facilitate the process, an electrically driven stirrer was employed. The decalcifying medium was changed every 36 hours. The first two changes of the acid solution, which was greyish in colour (dust from the mesh, etc.) was discarded without making any attempt to sediment the substances floating in it. It was assumed that such particles were most probably bits of pulp or soft tissue which still happened to be present, and were therefore discarded. Later, the hydrochloric acid solution was centrifuged and the particles collected and only the

clear decalcifying solution was discarded. The particles which floated off were kept separate from those still remaining in the flask since it was assumed that those particles which did not settle immediately were more decalcified.

A silver nitrate reaction carried out on the hydrochloric acid treated dentin fragments showed them to be relatively mineral free.

The decalcified dentin was washed with tap water and then with distilled water until almost chloride free with silver nitrate solution. This test was performed as an indication that the preparation was washed free of the acid.

The extraction of carbohydrates from the sample was carried out by the Glegg-Eidinger method using 0.5 N NaOH (20 g NaOH / litre) for four days with stirring. 250 ml of NaOH was used for 50 grams (wet) decalcified teeth. The NaOH supernatant was kept after centrifugation. The dentin was washed twice with aliquots of 0.5 N NaOH and the washings collected after centrifugation and put together with the first supernatant.

The sodium hydroxide extract solution was next neutralized to pH 7.1 (with Beckman pH meter) with concentrated acetic acid after which it was then decolourized by stirring with 6 grams of Hyflo-Super-Cel. There was not much coloured matter in the case of the dentin. The decolourizer was removed by centrifugation.

The straw coloured supernatant was evaporated on a steam cone to yield 200 ml of fluid from an original volume of 720 cc. The sediment found after reducing the volume was removed by centrifugation.

Two volumes of precipitating alcohol solution of 1% w/v potassium acetate and 1% v/v acetic acid in 95% ethanol were added with mixing to the neutralized extract. After allowing it to stand overnight in the refrigerator, the mixture was centrifuged and the precipitate called Fraction I.

The supernatant remaining after Fraction I was precipitated, and further fractionated by adding two more volumes of the same alcohol precipitating solution and allowed to stand overnight. The supernatant was removed by centrifugation and the precipitate obtained was called Fraction II. This second precipitate differed from the first in that it adhered to the walls of the container when allowed to stand overnight.

To purify Fraction I, the residue was dissolved in 50 cc of 10% CaCl_2 and centrifuged to remove any substance which did not dissolve. To the supernatant was added 10 cc of 4:1 chloroform-amy1 alcohol solution and the mixture stirred for 4 hours after which the protein containing gel was removed by centrifugation. Fraction I in the fluid was then precipitated by adding 1½ volumes of the same alcohol precipitating medium and stored overnight in the refrigerator.

After removing the alcohol solution by centrifugation, Fraction I was dissolved for the second time in 10% CaCl_2 solution. This was followed by adding 1 gram of Lloyd's reagent (deproteinization) and again stirred for 4 hours and centrifuged. Fraction I was finally precipitated with the alcohol solution, washed in absolute ethanol and then ether and dried.

To purify Fraction II, the same manipulations were carried out with the exception of omitting the use of 10% CaCl_2 solution; instead, distilled water was used.

The chromatographic isolation of hexoses was carried out after hydrolysis of the 2 fractions in the presence of a cation exchange resin, followed by ascending paper chromatographic separation of the hydrolysate in a medium consisting of n-butyl alcohol, pyridine and distilled water (Glegg and Eidinger. Anal. Chem., in press, 1955).

Results (Table I and Fig. 1)

Fraction I was found to contain mainly glucuronic acid and its lactone, (with traces of galactose, mannose and at least one unidentified spot). Fraction II was composed of galactose, glucose, mannose, fucose, glucuronic acid and its lactone.

Discussion and Conclusion

Two carbohydrate fractions were extracted from dentin by precipitation of an alkaline extract using two different concentrations of alcohol. The first fraction is rich in glucuronic acid and presumably corresponds to the acid mucopolysaccharide (described as chondroitin sulfuric acid) isolated by Hess and Lee (J. Dental Res. 31: 793, 1952). In vitro, this material produces an intense metachromasia of toluidin blue.

The second fraction is rich in galactose and contains small amounts of other hexoses and glucuronic acid. It is presumed to be responsible for the periodic acid-Schiff reaction.

II. Investigation of the C¹⁴ material taken up in dentin

Work carried out in collaboration with Greulich (Article 2) indicated that, at 4 hours after C¹⁴ administration, a radioactive material was detected by radioautography in pre-dentin. A day later this material appeared in the dentinal matrix, and as time passed, was found deeper and deeper within the dentin.

To find out whether this C¹⁴ labelled material consisted of carbohydrates it was decided to take the dentin of newborn rats given C¹⁴ and hydrolyse it for paper chromatographic analysis. To detect the radioactive substances present the paper chromatogram was placed in contact with an X-ray film and the location of the spots thus produced on the film were compared to those obtained with sugar carriers.

The detail of the technique is as follows:

The chromatograms of hydrolysates of C¹⁴-bicarbonate injected rat dentin were juxtaposed onto Kodak no screen X-ray film and stored in a metal cassette in a cold room. The films were kept dry by sealing the cassettes in a plastic bag. After 7½ months' exposure in the cold room, they were developed. It was expected that the radioactivity on the chromatograms would reduce the silver grains in the X-ray film emulsion, thus producing a greying or blackening over areas which contained any radioactivity.

None of the three visible spots on the film corresponded to any of the known carbohydrates. The same results were obtained with the dentin of rats sacrificed either 11 or 36 hours after injection. Some of the radioactivity was also retained on the resin used for hydrolysis as most amino acids are known to do.

It was tentatively concluded that either no carbohydrates were formed from the injected C¹⁴ or they were formed in too small amounts to be detected by this method. It appears more likely that the C¹⁴ has been incorporated into the amino acids of the protein of the dentin, that is collagen.

III. Articles submitted for publication

Article 1. Y. Kumamoto and C. P. Leblond. Radioautographic study of mineralization of growing teeth with labelled calcium. (Submitted for publication in the Journal of Dental Research.)

Article 2. R.C. Greulich and C. P. Leblond. Radioautographic visualization of the formation and fate of the organic matrix of dentin. Journal of Dental Research, 1954. (Accepted)

Article 3. C.P. Leblond, L.F. Belanger and R.C. Greulich. Formation of bones and teeth as visualized by radioautography. Annals of the New York Academy of Sciences (in press).

TABLE IData on Human Teeth Carbohydrates

Weight of dry powdered whole dentin	78.5 grams
Weight of dry decalcified dentin	21.49 grams
Fraction I	34.7 mg
Fraction II	51.3 mg

L-7

FRACTION I

FRACTION II

MARKERS

GLUCURONIC
LACTONE

FUCOSE

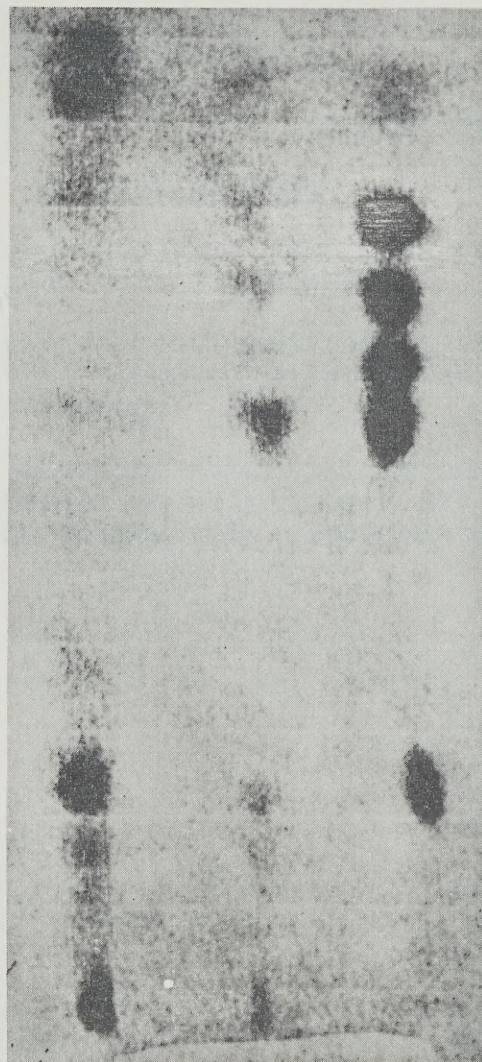
MANNOSE

GLUCOSE

GALACTOSE

GLUCURONIC
ACID

ORIGIN



APPENDIX M

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Electropotentials of dental alloys.

Grantee: H. R. MacLean, S. G. Davis,
University of Alberta.

Project No.: DR-43

Amount of Grant: \$250.00

SUMMARY OF WORK

Further measurements have been made on amalgam fillings using the vacuum tube electrometer.

Gold fillings have been prepared in the plastic holders. The potential that is produced between these and the various amalgams will be measured.

A method of measuring potential by discharging a condenser through a galvanometer has been set up and will be used along with the vacuum tube electrometer.

APPENDIX N

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: A cephalometric study of maxillary growth as related to statural growth.

Grantee: Dr. M. R. Culbert,
University of Toronto.

Project No.: DR-55

Amount of Grant: \$175.00

SUMMARY OF WORK

Permission was obtained from Dr. Meredith and Dr. Hixon of the University of Iowa to extract figures from the longitudinal anthropological study that has been under way for some years at that school. From a sample of 31 males and 32 females, measurements of maxillary antero posterior lengths were recorded from the Serial Cephalometric X-Ray head plates (semi-annually from 4 to 12 years). Serial Statural dimensions were also taken of the same sample group for comparison and statistical analysis.

These data are now under study and a final report will be forthcoming as soon as time permits.

APPENDIX O

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1954

Subject: Study of human dental pulp vessels by X-ray microarteriographic methods.

Grantee: R. L. deC. H. Saunders,
Dalhousie University.

Project No.: DR-51

Amount of Grant: \$1,870.00

SUMMARY OF WORK

Since February 1954, a satisfactory technique has been developed which permits the injection of a radiopaque agent into the dental pulp vessels, and their demonstration by microradiographic methods. In addition a routine radiographic procedure has been instituted which soon indicates the suitability of the injected tooth for further microscopic or microradiographic studies.

The general vascular pattern of the dental pulp has been demonstrated in nearly all the teeth of the adult dentition, and photographic enlargements made of the microarteriograms. These illustrate the disposition of the vessels in both longitudinal and transverse section.

Embryological and histological preparations have been studied with a view (a) to establishing the mode of development of the dental pulp vessels, (b) determining the structural characteristics and disposition of the intradental vasculature with a view to correlation with microradiographic appearances, and (c) elucidating the intradental and periodental vascular patterns detected microradiographically in the developing tooth.

Recently these microradiographic methods have demonstrated the existence of an intra-dental and peri-dental vascular net in and about the developing tooth of both the primary and secondary dentition. The microradiographic studies of the dental pulp vessels have now been extended to the investigation of both the developing and mature tooth. Various intra-dental factors appear responsible for non-filling of the vascular pattern in some teeth and are consequently being studied with a view to overcoming them.

APPENDIX P

Subject: A study of the development of occlusions on primitive diets.

Dr. E. E. Johns reported that he had spent three months with Dr. R. E. Moyers in Domini, a small village about 45 miles north of Athens, Greece. The inhabitants, who numbered about 750, lived under the most primitive conditions and while some were unco-operative, about 150 families were studied during the course of the survey. A diet survey was made in each of these homes during the first month; olive oil, beans, lentils and principally, black bread, formed that diet of the people surveyed and owing to the crude baking methods used, their bread contained an extremely high percentage of grit.

None of the people studied in the survey had ever had any dental care whatsoever and some time was spent in charting cavities and orthodontics in Domini and the neighbouring villages. Impressions were taken of all types of occlusion according to age groups and it was found that while 50 - 75% of children in Canada have some form of malocclusion, only 3% of the children studied in Greece showed any evidence of malocclusion. Moreover, while the teeth of children here show very little, if any, wear, the teeth of the Domini children showed terrific wear on the end-to-end bite. This extreme wear was not accompanied by decay, however, since the food did not collect on the teeth.

Dr. Johns stated that the teeth of Domini children of 4 - 5 years of age showed a normal adult relationship of the molars, something which is not found in Canadian children until the deciduous molars are lost. This relationship permits the lower mandible to come ahead earlier, and is normally not evident in Canadian children until they reach the age of about 14 years.

Dr. Johns also reported that children in Domini did not have the habit of sucking their thumbs, another cause of malocclusion. While he did not agree with those who believe that thumb-sucking has its origins entirely in some psychological quirk, Dr. Johns felt that the lack of it in Domini children was due not so much to the fact that their bread afforded them something to chew on as to the fact that they were too busy, since even the very young children were expected to do some type of work.

It was thought possible that the lack of malocclusions might be a racial characteristic, but an examination of Athenian children who were normally on a soft diet similar to our own showed that their teeth were much like those of Canadian children. It was therefore apparent that the abrasive diet and/or the vigorous chewing were responsible. It is hoped that an examination of primitive skulls available in various museums may throw some further light on the subject; conclusive evidence is difficult to find, however, for although there is no lack of skulls available for study, it is difficult to obtain skulls with enough teeth.

Dr. Johns reported that a control group had been organized in Burlington; the teeth of the children in the group are being examined each year, and an attempt is being made to have the children chew special abrasive gums in order to try and reconstruct the occlusions. One of the problems, here, of course, is to ensure the co-operation of the children.

In conclusion, Dr. Johns stated that it is still not clear whether the occlusion is the result of abrasive action or of vigorous chewing, or both, but it is hoped that further studies of primitive skulls, the control group at Burlington and experimental animals will eventually yield conclusive results.

APPENDIX Q

PAPERS PUBLISHED

<u>Committee Number</u>	<u>Title and Author</u>	<u>Reference</u>
1	New aspects of periodontal research, by H. K. BOX	Presented at National Convention of Canadian Dentists, Toronto, 29 May, 1946 (App. "C", 5th Mtg. A.C.D.R., 25 Feb., 1947).
2	Concerning the pathogenesis of periodontal disease, by H. K. BOX	J. Periodontology, 19:11-20. 1948. (App. "G", 7th Mtg. A.C.D.R., 2 Mar., 1948).
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J. J. RAE and C. T. CLEGG	J. Dent. Res. 27: 52-53. 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J. J. RAE and C. T. CLEGG	J. Dent. Res. 27: 54-57. 1948.
5	An improved method for processing acrylic dentures, by D. R. CHRISTIE	J. Can. Dent. Assoc., 14: 303-317. 1948. (App. "R", 8th Mtg. A.C.D.R., 26 Oct., 1948).
6	The therapeutic properties of periodontal cement packs, by W. J. LINGHORNE and D. C. O'CONNELL	J. Can. Dent. Assoc., 15: 199-205. 1949.
7	Phosphatase in saliva, by J. T. DENTAY and J. J. RAE	J. Dent. Res., Feb. 1949. 4 pp.
8	The treatment of acute oral Vincent's infection, by W. J. LINGHORNE and D. J. STEDMAN	J. Can. Dent. Assoc., July, 1949. 6 pp.
9	A comparison of nine local anaesthetics, by H. S. HAMILTON, B. A. WESTFALL and J. K. W. FERGUSON	J. Pharmacol. Expt. Therap. 94: 299-307. 1948.
10	New methods of repairing acrylic dentures without distortion, by D. R. CHRISTIE	J. Can. Dent. Assoc., 15: 582-590. 1949.

<u>Committee Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D. E. R. COGGLES, O. J. WALKER and H. R. MACLEAN	J. Can. Dent. Assoc., 16: 75-82. 1950
12	The relation between buffering capacity, viscosity and lacto-bacillus count of saliva, by J. J. RAE and C. T. CLEGG	J. Dent. Res. 28: 589-593. 1949.
13	Mineralization of the growing tooth as shown by radiophosphorus autographs (17692) by L. F. BELANGER and C. P. LEBLOND	Proc. Soc. Expt. Biol. Med. 73: 390-391. 1950.
14	Radio-autographic visualization of bone formation in the rat, by C. P. LEBLOND, G. W. WILKINSON, L. F. BELANGER and J. ROBICHON	Am. J. Anat. 86: 2. 1950.
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH	Can. J. Research, F. 28: 227-233. 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W. J. LINGHORNE and D. C. O'CONNELL	J. Dent. Res. 29: 419-428. 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH	J. Can. Dent. Assoc., Jan., 1951.
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A. D'IORIO and J. P. LUSSIER	Rev. Canadienne de Biol. 10: 175-180. 1951.
19	Relining acrylic dentures without distortion, by D. R. CHRISTIE	J. Can. Dent. Assoc., July, 1951.
20	Acrylic fillings - General comments and some experimental data, by D. R. CHRISTIE	J. Can. Dent. Assoc., 17: 427-435. 1951.
21	Ammonia production in saliva, by R. M. BALLANTYNE, C. T. CLEGG, J. J. RAE and F. H. LAWFORD	J. Dent. Res. 30: 385-387. 1951

Committee
Paper No.

Title and Author

Reference

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| 22 | Studies in the regeneration and reattachment of supporting structures of the teeth. II. Regeneration of alveolar process, by W. J. LINGHORNE and D. C. O'CONNELL | J. Dent. Res. 30: 604-614
1951. |
| 23 | Radioactive isotopes in dental research by J. P. LUSSIER and A. D'IORIO | The Brit. Dent. Annual,
1952. |
| 24 | Determination of fluoride in water by the aluminum-hematoxylin method, by MARGARET J. PRICE and O. J. WALKER | Anal. Chem., 24: 1593.
1952. |
| 25 | Autoradiographic detection of S^{35} in the membranes of the inner ear of the rat, by L. F. BELANGER | Science, 30 October, 1954.
118: 520-521 |
| 26 | The motile non-sporulating anaerobic rods of the oral cavity, by J. B. MACDONALD | University of Toronto
Press, Toronto, 1953. |
| 27 | Motility in a species of non-flagellated bacteria (20677), by J. B. MACDONALD, M. L. KNOLL and R. M. SUTTON (Introduced by J.K.W. FERGUSON) | Proc. Soc. Exp. Biol.
and Med. 1953, vol. 84,
459-462. |
| 28 | The periodontium and salivary glands in the alarm reaction, by G. SHKLAR and I. GLICKMAN | J. Dent. Res. 32: 773-778,
1953. |
| 29 | Autoradiographic visualization of S^{35} incorporation and turnover by the mucous glands of the gastro-intestinal tract and other soft tissues of rat and hamster, by L. F. BELANGER | Anat. Rec. 118: 755-772,
1954. |
| 30 | Autoradiographic visualization of the entry and transit of S^{35} in cartilage, bone, and dentine of young rats and the effect of hyaluronidase <u>in vitro</u> , by L. F. BELANGER | Can. J. Biochem. & Physiol.
32: 161-169, 1954. |

Committee
Paper No.

Title and Author

Reference

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| 31 | Autoradiographic visualization with Ca^{45} of normal growth of the incisor of pigs and the effect of fluorine feeding, by L. F. BELANGER, W. E. LOTZ, W. J. VISEK and C. L. COMAR | Anat. Rec. 119: 53-70, 1954 |
| 32 | Fluorine balance studies of four infants, by M. P. HAM and M. DOREEN SMITH | J. Nutrition, 53: 215-223, 1954. |
| 33 | Fluorine balance studies of three women, by MARY P. HAM and M. DOREEN SMITH | J. Nutrition, 53: 225-232, 1954 |
| 34 | The effect of propylthiouracil on the development of molar teeth of rats, by K. J. PAYNTER | J. Dent. Res. 33: 364-376, 1954. |

APPENDIX R

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

List of Grantees and Subjects

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR 1	Active	Dr. J. J. Rae, Dept. of Chemistry, University of Toronto	Chemical mechanisms in dental caries
DR 2	Completed	Dr. G. H. W. Lucas, Dept. of Pharmacology, University of Toronto	A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia
DR 3	Completed	Dr. H. K. Box, Faculty of Dentistry, University of Toronto	A study of microorganisms found in deep period- ontal pockets and caries lesion as revealed by the electromicroscope
DR 4	Completed	Dr. H. K. Box, Faculty of Dentistry, University of Toronto	An investigation of packing material in the treatment of periodontal disease (continuation of in- vestigation previously carried on by Maj. Linghorne)
DR 5	Completed	Major R. L. Twible, Royal Canadian Dental Corps (later Ontario Research Foundation)	To improve certain pro- perties of the acrylic resin denture base and to effect an economy in denture production
DR 6	Completed	Brig. E. M. Wansbrough, Royal Canadian Dental Corps	To study the effects of altitude acceleration and deceleration on oral tissues
DR 7	Completed	Dr. J. S. Bagnall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries
DR 8	Completed	Dr. A. D. A. Mason, Faculty of Dentistry, University of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR 9	Completed	Dr. O. J. Walker Dept. of Chemistry, Dr. H. R. MacLean, Faculty of Dentistry, University of Alberta	An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry
DR 10	Completed	Dr. G.M. Macdonald, Queen Mary Veterans' Hospital, Montreal	Facial prostheses
DR 11	Completed	Dr. R. J. Godfrey, Faculty of Dentistry, University of Toronto	An anatomical, histological and physiological study of the role of the Condyle in mastication
DR 12	Completed	Dr. A. E. R. Westman, Ontario Research Foundation	Investigation of cements for methacrylate inlays
DR 13	Active	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluoride content of dentine, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford
DR 14	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries
DR 15	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	A comparison of the patterns of eruption of the deciduous teeth in man with rate of growth of the dental arches
DR 16	Completed	Dr. C. H. Best, Dept. of Medical Research, University of Toronto	Studies in vitro of certain fungus-like organisms found in periodontal diseases
DR 17	Completed	Dr. R. F. Shaner, Dept. of Anatomy, University of Alberta	An investigation of the effects of solutions used in operative dentistry on the vital pulps of teeth

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR 18	Completed	Dr. H. K. Box, Faculty of Dentistry, University of Toronto	Experimental production of dental caries
DR 19	Completed	Dr. E. A. Sellers, Dept. of Pharmacology, University of Toronto	Tissue toxicity and efficiency of local anaesthetics for topical application and nerve block in dentistry
DR 20	Completed	Dr. Jules Thébaud, Faculty of Dental Surgery, University of Montreal	Improvement of subgingival curettage and surgical techniques used in the treatment of pyorrhea alveolaris
DR 21	Completed	Dr. A. E. R. Westman, Ontario Research Found- ation	Investigation of repair techniques for metha- crylate dentures
DR 22	Active	Dr. C. H. Best, Dept. of Medical Research, University of Toronto	An investigation of the mechanism of repair of the supporting struc- tures of the teeth and factors affecting the reparative process
DR 23	Active	Dr. C. P. Leblond, Dept. of Anatomy, McGill University	Entry of phosphate, car- bonate and calcium ions into teeth, as shown with the help of radio- active elements
DR 24	Completed	Dr. C. H. M. Williams, Faculty of Dentistry, University of Toronto	The effects of hard and soft diets on the supporting structures of the teeth
DR 25	Completed	Dr. A. E. R. Westman, Ontario Research Found- ation	Investigation of methods for the use of methyl methacrylate in direct dental fillings
DR 26	Completed	Dr. R. E. Moyers, Faculty of Dentistry, University of Toronto	A study of the normal and abnormal physiologic forces of the oral cavity

<u>Grant No.</u>			<u>Subject</u>
R 27	Completed	Dr. J. W. Abbiss, Faculty of Dentistry, Dr. A. G. Nutlay, Faculty of Dentistry, Dalhousie University	A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth
R 28	Active	Dr. O. J. Walker, Dept. of Chemistry, University of Alberta	Investigation of methods for destroying the organic matter in teeth bones and other materials prior to determination of fluorine content
R 29	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Biological absorption of fluorine
R 30	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	The study of the closure of space following premature loss of deciduous teeth
R 31	Completed	Dr. L. B. Jaques, Dept. of Physiology, Mr. G. J. Millar, Dept. of Physiology, University of Saskatchewan	An investigation of nerve block
R 32	Completed	Dr. A. W. Ham, Dept. of Anatomy, University of Toronto	Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structures of the growing incisor teeth of animals
R 33	Completed	Dr. H. A. Hunter, Faculty of Dentistry, University of Toronto	A study of calcifying potential of calcium oleate on the dental pulp
R 34	Completed	Er. A. D'Iorio, Dept. of Physiology, University of Montreal	Studies of calcium metabolism in tooth with the aid of Ca ⁴⁵
R 35	Active	Dr. J. B. Macdonald, Faculty of Dentistry, University of Toronto	Bacteriology of periodon- tal disease I. Experi- mental fusospirochetal infection with mixtures of pure culture

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
R 36	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Demineralization of hard tissues as bone and teeth by organic che- lating agents
R 37	Active	Dr. H. Jenkins, Dept. of Orthodontics, University of Toronto	Further studies in the electromyography of the head, face and neck
R 38	Completed	Dr. K. J. Paynter, Faculty of Dentistry, University of Toronto	The effects of the endo- crines on the teeth and jaws. II. Further studies on the effect of thiouracil on the de- velopment of molar teeth of rats
R 39	Completed	Dr. A. G. Racey, Faculty of Dentistry,	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine de- ficiency on the teeth and surrounding struc- tures of dogs
R 40	Completed	Dr. L. A. Funt, Faculty of Dentistry, University of Toronto	Effect of tooth eruption and function on the growing of the mandible and facial complex on cats
R 41	Completed	Dr. Harvey Jenkins, Faculty of Dentistry, University of Toronto	A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions (After Downs (1))
R 42	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxi- dation-reduction indica- tors
R 43	Active	Dr. S. G. Davis, Dept. of Chemistry, Dr. H. R. MacLean, Faculty of Dentistry, University of Alberta	Electro potentials caused by dental alloys

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
R 44	Completed	Dr. Arthur Harry Craven, Faculty of Dentistry, McGill University	Facial and cranial growth patterns in the rat as revealed by vital staining with alizarine red S
R 45	Completed	Dr. R. E. Moyers, Faculty of Dentistry, University of Toronto	Study of the incidence of malocclusion
R 46	Completed	Dr. H. Jenkins, Dept. of Orthodontics, University of Toronto	Study of treated cases
R 47	Completed	Dr. H. A. Hunter, Faculty of Dentistry, University of Toronto	The pathological characteristics of experimental fusospirochetal infections
R 48	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	The effect of topical application of silicones in protecting teeth against acid solutions in vitro and dental caries produced in vitro
R 49	Completed	Dr. A. H. Craven, Faculty of Dentistry, University of Toronto	A recording device to determine postural changes of the head in the horizontal plane
R 50	Active	Dr. J. B. Macdonald, Faculty of Dentistry, University of Toronto	The cultivation and characteristics of <u>Bacteroides nigrescens</u>
R 51	Active	Dr. R.L. deC. H. Saunders, Prof. of Anatomy, Dalhousie University	Study of human dental pulp blood vessels by X-ray micro-arteriographic methods
R 52	Active	Dr. L. F. Bélanger, Dept. of Histology and Embryology, University of Ottawa	Mechanism of bone and tooth formation in the light of autoradiography and histochemistry
R 53	Completed	Dr. C. B. Weld, Dept. of Physiology, Dalhousie University	The macrophages in the pulp tissue of the rat incisor and lower jaw

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR 54	Active	Dr. B. N. Kröpp, Faculty of Medicine Queen's University	Development of a rapid method for grinding thin sections of plastic-embedded teeth
DR 55	Active	Dr. M. R. Culbert, Assistant Professor of Orthodontics, University of Toronto	A cephalometric study of maxillary growth as related to statural growth
DR 56	Active	Dr. D. J. E. Mitchell, Dept. of Orthodontics, University of Toronto,	Comparative study of palatal height, width and length in ancient and modern crania
DR 57	Active	Dr. G. Nikiforuk, Associate Professor of Pedodontics, University of Toronto	The amino acid composition of enamel protein using paper chromatography

APPENDIX S

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointment

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Faculty of Dentistry,
University of Toronto,
Toronto 5, Ontario. | 31 March 1957 |
| 2. Dr. E. W. R. Steacie, <u>ex officio</u>
President,
National Research Council,
Ottawa 2, Ontario. | - - |

Representing the Dental Profession

- | | |
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| 3. Dr. J. S. Bagnall,
Dean, Faculty of Dentistry,
Dalhousie University,
Halifax, N. S. | 31 March 1955 |
| 4. Dr. C. D. Husband,
Suite 110, Metro Block,
Red Deer, Alberta. | 31 March 1956 |
| 5. Dr. Jean-Paul Lussier,
Assistant Professor,
Faculty of Dental Surgery,
University of Montreal,
Montreal, P. Q. | 31 March 1957 |
| 6. Dr. R. H. McDougall,
1505 Hampshire Road,
Victoria, B. C. | 31 March 1955 |
| ★ 7. Dr. A. G. Racey,
Associate Professor of Oral Pathology,
McGill University,
Montreal, P. Q. | 31 March 1956 |
| 8. Dr. C. H. M. Williams,
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Toronto 5, Ontario. | 31 March 1957 |

Representing the Royal Canadian Dental Corps, Department of National Defence

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| ★ 9. Brig. E. M. Wansbrough, <u>ex officio</u> ,
Director General of Dental Services,
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Ottawa, Ontario. | - - |
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Representing the Division of Dental Health,
Department of National Health and Welfare.

10. Dr. H. K. Brown, ex officio
Chief, Dental Health Division,
Dept. of National Health and Welfare,
Ottawa, Ontario. - -

Representing Dental Research,
Department of Veterans Affairs

11. Dr. L. A. Kilburn, ex officio,
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Head of the Department of Pharmacology,
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13. Dr. J. M. Beveridge, 31 March 1956
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Queen's University,
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14. Dr. A. C. Burton, 31 March 1956
Head of the Dept. of Biophysics,
University of British Columbia,
Vancouver, B. C.
15. Dr. H. Copp, 31 March 1956
Professor of Physiology, Faculty of Medicine,
University of British Columbia,
Vancouver, B. C.
- * 16. Dr. A. W. Ham, 31 March 1957
Professor of Anatomy,
Faculty of Medicine,
University of Toronto,
Toronto 5, Ontario.
17. Dr. Edouard Pagé, 31 March 1955
Director, Department of Nutrition,
Medical School, Laval University,
Quebec, P. Q.
- * 18. Dr. J. B. Marshall, Secretary, - -
National Research Council,
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